

REPRODUCTIVE ECOLOGY AND POPULATION GENETICS OF HAWAIIAN WILIWILI,
ERYTHRINA SANDWICENSIS (FABACEAE)

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF
HAWAI'I AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF

MASTER OF SCIENCE

IN

BOTANY (ECOLOGY, EVOLUTION, AND CONSERVATION BIOLOGY)

AUGUST 2018

By

Emily F. Grave

Thesis Committee:

Tamara Ticktin (Chairperson)

Curt Daehler

Cliff Morden

Daniela Elliot

Keywords: dry forest, pollination biology, population genetics, conservation biology, *Erythrina*

DEDICATION

I would like to dedicate this work, and resulting thesis, to my late mother Barbara Jean Grave. My true love of nature was fostered through my first teacher, my mom, who never stopped learning and teaching me about the beauty, wonder, and significance of the world around us. And to my son, Sebastian, for whom I hope to bestow upon my fascination with our enigmatic ecosystems.

ACKNOWLEDGMENTS

I would like to take this opportunity to thank the many people who have sacrificed their time to advance this project and its several components. My committee members, Tamara Ticktin, Curt Daehler, Daniela Elliot, and Cliff Morden have been incredibly insightful and supportive, and have helped propel this study into fruition. My field assistant and partner, Tim Kroessig, was instrumental in formulating the basis behind this research. From realizing the importance of the research, to the proposal and permitting, to hours of field work, and onward through the data collection phase, Tim helped make this study a successful reality. His ideas and innovations greatly strengthened the results of the research. I am very grateful to my lab mates: Reko Libby, Zoe Hastings, Georgia Fredeluces-Hart, Ashley McGuigan, Gioconda López, and Leo Beltran (and Solomon Champion and Miles Thomas as well), who spent several hours in (and out of) the field with me. These generous people helped build the hanger contraptions, conducted pollinator observations, took notes, collected wire and seeds, carried the ladder up and down the hill at Mākua Kea‘au, helped discover R packages that could handle my data, and provided overall emotional support.

I would like to thank my wonderful family: Terry Grave, Elizabeth Grave, Jonathan Partch, Cleo Partch, and my son Sebastian Grave Tobias. Your influence and support has been monumental in my efforts to complete this study. Thank you for continuously listening to my remonstrations, for your wisdom and edits, and reassuring me along this arduous journey.

Many people were involved in providing me with background information on the species, its habitat, population locations, key researchers and scientists to network with, lab work, species identification, permitting, and general support throughout the project. They were: Shad Kane (Kalaeloa Heritage Park), Naomi Hoffman (City and County, Honolulu), Matt Keir, Jill Wagner, Jen Lawson, Bruce Koebele, Art Medeiros, Chuck Chimmera, Karl Magnacca, Bill Garnett, Mitsuko Yorkston, Bethany Davis, Susan Ching, Ryan Peralta, Tamara Ticktin’s lab, my cohort, and co-workers at Lyon Arboretum.

I would like to thank the generous donors who have helped me financially throughout this journey: Mrs. Karen V. Howell (Beatrice Krauss Fellowship Fund in Botany), Mrs. Florence K.

Lamoureux (Charles H. Lamoureux Fellowship in Plant Conservation Fund), Ms. Masayo A. Char (Winona Pi'ilani Char Scholarship), and the Botany Department of UH Mānoa.

Finally, I would like to thank everyone in the Department of Botany at UH Mānoa. I have never experienced such kindness and helpfulness from professors and professionals in this field. Thank you all for sharing your knowledge with me. This thesis would not be a reality without each and every one of you. Mahalo nui loa.

ABSTRACT

Hawaiian dry forests are severely endangered with little habitat remaining and many threatened or endangered species. Wiliwili, or *Erythrina sandwicensis* (Fabaceae), is among the most iconic dry forest trees. This research focuses on the reproductive ecology, population genetics, and regeneration of wiliwili by addressing: 1) Who are the current flower visitors? 2) What is the effect of different pollination treatments on the number, size, and viability of wiliwili seeds and seedlings? 3) What is the genetic relationship among wiliwili populations? and 4) What is the regeneration status of populations on O‘ahu and Hawai‘i islands? I found that all floral visitors were non-native species. Outcrossed pollination treatments produced significantly more fruit and seeds than any other treatment. Few seedlings and saplings were found in any population, and populations were genetically distinct across islands. Outplanting efforts can increase recruitment by enhancing the potential for outcrossing and increasing the number of seedlings and saplings.

TABLE OF CONTENTS

DEDICATION.....	ii
ACKNOWLEDGEMENTS.....	iii
ABSTRACT.....	v
LIST OF TABLES.....	viii
LIST OF FIGURES.....	x
CHAPTER 1. OVERVIEW OF WILIWILI.....	1
Introduction.....	1
Research Questions.....	5
Hypotheses.....	6
CHAPTER 2. POLLINATION BIOLOGY OF <i>ERYTHRINA SANDWICENSIS</i>	7
Abstract.....	7
Introduction.....	7
Methods.....	10
Study species.....	10
Study sites.....	11
Experimental design.....	11
Statistical analyses.....	14
Results.....	14
Nectar.....	15
Floral visitors observed on wiliwili.....	15
Effects of pollination treatment and site.....	17
Discussion.....	18
Nectar.....	18
Floral visitors.....	19
Breeding system.....	20
CHAPTER 3. POPULATION GENETICS OF HAWAIIAN WILIWILI.....	30

Abstract.....	30
Introduction.....	30
Materials and Methods.....	33
Study species.....	33
Plant materials.....	34
DNA extraction.....	36
SRAP amplification.....	36
SRAP analysis.....	38
Data analyses.....	39
STRUCTURE analysis.....	39
Results.....	40
Polymorphism and genetic diversity.....	40
Similarity matrix.....	41
Principal coordinate analysis.....	41
STRUCTURE.....	42
Discussion.....	42
CHAPTER 4. DEMOGRAPHIC STRUCTURE AND PATTERNS OF SEEDLING RECRUITMENT IN <i>ERYTHRINA SANDWICENSIS</i>	50
Abstract.....	50
Introduction.....	50
Methods.....	53
Study sites.....	53
Experimental design.....	53
Results.....	54
Discussion.....	57
CHAPTER 5. CONSERVATION OF <i>E. SANDWICENSIS</i>	61
APPENDIX.....	63
LITERATURE CITED.....	66

LIST OF TABLES

2.1. Nectar sucrose concentration (%) by time of day.....	15
2.2. Germination day and leaf area experiments (by treatment).....	18
3.1. Collections of <i>Erythrina sandwicensis</i> used from each population for SRAP analysis.....	35
3.2. The forward and reverse SRAP primers used for this study.....	37
3.3. SRAP primers screened (and selected) for this study.....	38
3.4. Calculated percentage of polymorphic loci and expected heterozygosity (H_e).....	40
3.5. Genetic similarities among islands.....	41
4.1. Site-specific environmental factors (no scale) and mean of abiotic/biotic factors.....	55
4.2. Results of wiliwili demographic study.....	56
A1. Best fit model of the effects of pollination treatment and site on the number of fruit produced per flower.....	63
A2. Best fit model of the effects of pollination treatment and site on the number of seeds produced per flower.....	63
A3. Best fit model of the effects of pollination treatment and site on the number of seeds produced per fruit.....	63
A4. Best fit model of the effects of pollination treatment and site on the mass of the seed.....	63
A5. Best fit model of the effect of site and pollination treatment on the probability of germination.....	64

A6. Best fit model of the effect of site and mass on the probability of germination.....	64
A7. Best fit model of the effect of pollination treatment and site on the time to germinate.....	64
A8. Best fit model of the effect of pollination treatment and site on seedling growth rate.....	64
A9. Best fit model of the effect of mass, pollination treatment, and site on seedling growth rate.....	64
A10. Best fit model of the effect of pollination treatment on final seedling height.....	65
A11. Best fit model of the effect of pollination treatment and site on total leaf area.....	65
A12. Best fit model of the effect of pollination treatment and site on seedling basal diameter...	65
A13. Best fit model of the effect of mass on seedling height.....	65

LIST OF FIGURES

2.1. Labeled photo of wiliwili floral morphology.....	23
2.2. Invertebrate visitors to wiliwili flowers.....	23
2.3. Avian visitors to wiliwili flowers.....	24
2.4. Number of fruit set per flower as a function of pollination treatment and site.....	24
2.5. Number of wiliwili seeds set per flower, as a function of pollination treatment and site...	25
2.6. Number of seeds set per fruit as a function of pollination treatment and site.....	25
2.7. Effect of pollination treatment and site on seed mass.....	26
2.8. Probability of germination as a function of pollination treatment and site.....	26
2.9. Seedling growth rate (growth per day) as a function of pollination treatment and site.....	27
2.10. Effect of pollination treatment and site on seedling height 28 days after planting.....	28
2.11. Effect of pollination treatment and site on final seedling basal diameter 28 days after planting.....	28
2.12. Effect of seed mass on seedling height 28 days after planting.....	29
2.13. Summary of breeding system results.....	29

3.1. PCO based on SRAP marks for individuals of Hawaiian <i>Erythrina sandwicensis</i> from all islands.....	45
3.2. PCO based on SRAP marks for individuals of Hawaiian <i>Erythrina sandwicensis</i> from all island populations.....	46
3.3. PCO based on SRAP marks for individuals of Hawaiian <i>Erythrina sandwicensis</i> from Maui, Lana‘i, and Moloka‘i.....	47
3.4. PCO based on SRAP marks for individuals of Hawaiian <i>Erythrina sandwicensis</i> from Hawai‘i and Kaho‘olawe.....	48
3.5. Genetic STRUCTURE bar graph of 71 <i>Erythrina sandwicensis</i> individuals from all islands based on SRAP data.....	49
4.1. Graph of wiliwili demographic study.....	60

CHAPTER 1. OVERVIEW OF WILIWILI

Introduction:

Tropical dry forests are among the most imperiled ecosystems in the world. In Mesoamerica, less than two percent of these ecosystems are considered intact and worth preservation (Janzen 1988; Miles et al. 2006). A global assessment on the status of tropical dry forests by Miles et al. (2006) revealed that only a small portion of these ecosystems are designated as protected. In Hawai‘i, the pattern of loss is consistent with the global trend, with estimates of less than ten percent of these habitats still in existence (Merhoff 1996, Sakai et al. 2002). However, when these estimates are broken down by island, subtler and more concerning patterns emerge. Sakai et al. (2002) report that more than ninety-nine percent of lowland dry forest and shrubland have been completely degraded on the islands of O‘ahu, Moloka‘i, and Lana‘i, with Maui having lost more than ninety-eight percent. In comparison, the Big Island of Hawai‘i has retained about seventeen percent of their dry forest habitat (Sakai et al. 2002), which skews the state’s estimated total area of remaining dry forest. Over the last two hundred years, a number of pressures have resulted in the destruction, fragmentation and degradation of these ecosystems including: land development, fire, the introduction of non-native plant, animal, and insect species, and the loss of mutualisms including pollinators and dispersers (Bruegmann 1996; Cabin et al. 2000; Cox and Elmqvist 2000; Messing et al. 2007; Cordell et al. 2008). It is estimated that twenty percent of species with dry, lowland distributions in Hawai‘i have gone extinct (Sakai et al. 2002).

The flowering plants of Hawai‘i have experienced a great amount of speciation with about ninety percent of all taxa being endemic, or, found nowhere else in the world (Sakai et al. 2002; Ziegler 2002). Endemic species are far more likely to be at risk than indigenous species (Sakai et al. 2002). This is because of the archipelago’s geologic history and isolation from mainland continents, where species in Hawai‘i have evolved in the absence of pressures, such as parasites and herbivory, that indigenous species have evolved with (Cox and Elmqvist 2000; Ziegler 2002). It is not surprising that Hawai‘i has “more endangered species per square mile than any other place on Earth” (Messing et al. 2007). More than half of native Hawaiian plant

species (about sixty percent) are federally listed as endangered, and twenty-five percent of these species occur in dry lowland, or scrubland ecosystems (Bruegmann 1996; Sakai et al. 2002). A study by Pau et al. (2009) found that forty-five percent of taxa in these systems are at risk of becoming endangered. One of the most iconic species that occurs in these habitats is the Hawaiian wiliwili, Hawaiian Coral Tree, or *Erythrina sandwicensis* (O. Degener). Wiliwili is the only Hawaiian endemic species in a genus of one hundred fifteen species, and is a member of Fabaceae, or the legume family (Wagner et al. 1999).

Hawaiian wiliwili once dominated the landscape of lowland elevations, up to six hundred meters (Rock 1913; Medeiros et al. 2008). Its distribution is primarily restricted to the hottest, driest environments of the leeward sides on all of the main Hawaiian Islands (Rock 1913; Staples and Herbst 2005). It is a summer-deciduous tree, gaining its leaves in the wet season and shedding them right before flowering which usually occurs in early spring to July, when the tree is completely (or partially) leafless (Rock 1913; Staples and Herbst 2005). The swollen trunks of these trees are conspicuously colored yellow to orange, with prickles that protrude from the branches (Rock 1913; Staples and Herbst 2005). These trees reputedly have the lightest wood of any Hawaiian tree species which was traditionally used for buoyancy in fishing canoe outriggers ('ama), net floats, and containers, as well as for surfboards (papa he'e nalu) (Rock 1913; Medeiros et al. 1998; Staples and Herbst 2005). Bark of this tree was also used medically, and for charcoal dye-making (Medeiros et al. 1998). The flowers of wiliwili vary in an array of pastel colors, from creamy white and green to salmon-orange, with every color between (Medeiros et al. 1998; Staples and Herbst 2005) and were once called "tiger's claw" by island new-comers due to their claw-like form (Rock 1913). The seeds of wiliwili vary in color as well, from bright red to yellow, and are valued in the jewelry industry today, just as they were in the past (Rock 1913; Medeiros et al. 1998; Staples and Herbst 2005). As culturally significant and abundant as this tree was, it is much less common today than in the past (B. P. Koebele 2016, personal communication, 23 December). Currently, wiliwili is not listed as endangered but is at risk of endangerment, which Pau et al. (2009) attribute to having hermaphroditic, conspicuous flowers, dry fruit that require autochorous dispersal methods, and a large range over more than five islands.

There is very little recruitment observed in these remaining populations, if any, due to a number of limiting factors discussed below (Kaufman et al. 2014).

Assessing the demographic structure of a population can give insight into the status of its regeneration (Gurevitch et al. 2006). One such assessment elucidated that less than one percent of native dry forest species are naturally regenerating even in an area where invasive plant species and browsing ungulates were removed (Cordell et al. 2008). However, invertebrates may be important limiting factors, and the introduction of exotic insects is very difficult to manage. Although largely unseen to the human eye, the effects they have on the native flora can be substantial (Ceballos et al. 2002; Messing et al. 2007; Hue et al. 2008; Medeiros et al. 2008; Messing et al. 2009; Gumovsky and Ramadan 2011; Bell et al. 2013; Kaufman et al. 2014). An estimated twenty new arthropods are introduced, and subsequently become established in Hawai'i every year (Messing et al. 2007). For wiliwili, some of these introductions have been devastating. The host-specific Erythrina Gall Wasp, *Quadrastichus erythrinae*, was discovered on the island of O'ahu in April 2005 where it spread to adjacent islands in just four months (Bell et al. 2013). This pest causes substantial defoliation and, in the worst scenarios, tree mortality in just a few months (Kaufman et al. 2014). The biological control agent (*Eurytoma erythrinae*), released as a parasitoid of this wasp in 2008, has been somewhat successful in mitigating these effects (Messing et al. 2009; Bell et al. 2013; Kaufman et al. 2014). However, a 2012 state-wide census revealed that an estimated 30-35% percent of wiliwili trees had died due to the gall wasp attack (Kaufman et al. 2014).

Another introduced arthropod to the Hawaiian Islands that could limit recruitment of wiliwili is the African bruchid beetle, *Specularius impressithorax*. In 2001, the beetle was observed and collected in Makaha Valley on O'ahu island. It took only two years for this seed-predating beetle to become established on every main Hawaiian island (Medeiros et al. 2008). A study by Medeiros et al. (2008) found that the bruchid beetle accounted for seventy-seven percent of mean seed crop loss in twelve wiliwili populations, on six of the main islands, in the first three years since its introduction. This is because one single larva can reduce the germination rate of the seed in a pod by ninety-seven percent (Medeiros et al. 2008). A biological control endoparasitoid, *Entedon erythrinae*, probably accidentally arrived in the islands

with the bruchid beetle. However, with a low rate of recovery for this parasitoid, it may not be an effective control agent in this new, Hawaiian environment (Kaufman et al. 2014). The gall wasp and bruchid beetle together may have catastrophic impacts on reproduction in wiliwili populations (Gumovsky and Ramadan 2011).

There are still other factors that might be influencing the level of regeneration in many wiliwili populations. Invasive grasses inhibit the germination of seeds by covering the landscape and preventing sufficient light levels from reaching the soil (Cabin et al. 2002). These grasses make great fuel for fires that destroy native dryland plants and habitats, and they are able to regenerate after such disturbances, actually thriving in a burnt landscape (Cabin et al. 2002). Grasses and other invasive plant species tend to alter the natural energy fluxes within ecosystems as well as the quality of available resources and habitat. This can disrupt the mutualisms that have evolved between plants and animals in these systems (Traveset and Richardson 2006). These mutualistic mechanisms governing pollination and seed dispersal are fragile and thought to be “the most vulnerable processes in the life cycle of plants” (Neushulz et al. 2016), as they represent the most essential precursors for regeneration and maintenance of structure (Traveset and Richardson 2006). In Hawai‘i, many of these interactions have not been studied, and knowledge of a species’ reproductive ecology is vital for the persistence of regenerating populations (Gardener and Daehler 2006).

The breeding system, which directly affects the regeneration of wiliwili, has yet to be studied in this species. Pollination ecology studies can yield significant insight on potential regeneration bottlenecks for species at risk, especially if native pollinators are thought to have declined or are no longer present (Kearns and Inouye 1993; Gardener and Daehler 2006). Although there is a body of literature on the pollination ecology of other species in the genus *Erythrina*, there has been very little research conducted with wiliwili. Pollinators of the genus range from hummingbirds and passerine birds, to bees and, possibly, other insects and even bats, but the genus is thought to be bird-adapted (Baker and Baker 1983; Neill 1988; Bruneau 1997; Etcheverry et al. 2005, 2012; Galetto et al. 2014). Because of this early adaptation in its evolutionary history, the genus is thought to be largely xenogamous, or self-incompatible (Etcheverry et al. 2005; Etcheverry et al. 2012). Species that are highly xenogamous often

experience limitations in reproductive success due to constrained pollen availability, where trials proved that higher fecundity results from artificially outcrossed pollination (Burd 1994). One species (*E. crista-galli*) will set seed in autogamous (selfing) experiments successfully (Galetto et al. 2014), though the germination rates of these seeds were low (Etcheverry et al. 2005). Studies have shown that, even in partially self-compatible species, the probability of higher fruit and seed set and the chance that resulting seeds will have more mass, is greater with the application of outcrossed pollen (Jordano 1993; Rojas-Sandoval and Meléndez-Ackerman 2009).

Birds are known to be attracted to red or orange flowers that produce nectar (Gurevitch et al. 2006), like those of *Erythrina* species. The flowers of wiliwili, however, vary in color as mentioned above. Because corolla color can affect the guild of floral visitors (Gurevitch et al. 2006), it is conceivable that the flowers of wiliwili are not exclusively bird-pollinated, but may be host to a new suite of visitors. A non-native, yellow-faced bee species (*Hylaeus strenuus*) has been observed visiting flowers (K. N. Magnacca 2017, pers. comm., 2 February) and vertebrate visitors to the flowers of wiliwili include non-native birds such as red-vented bulbuls (*Pycnonotus cafer*), common mynas (*Acridotheres tristis*), and Japanese white-eyes (*Zosterops japonicus*) (Staples and Herbst 2005).

Research Questions:

In an effort to collect data that produces useful, informative results for conservation management of *Erythrina sandwicensis*, I addressed the following questions:

1. What species are the current flower visitors of wiliwili?
2. What is the effect of different pollination treatments on the number, size, and viability of wiliwili seeds and seedlings?
3. Do the number and size of seeds produced vary across sites?
4. What is the genetic relationship among wiliwili populations?
5. What is the regeneration status of wiliwili populations on O‘ahu and Hawai‘i?
6. What are the limiting factors of recruitment?

Hypotheses:

H₁. Due to the loss of possible native pollinators, I hypothesized that wiliwili is visited by a novel guild of potential pollinators to the genus.

H₂. Because of the tendency for species in this genus to be self-incompatible, I hypothesized that seeds produced from spatially distant outcrossing treatments would have higher seed and fruit set, greater seed mass, and higher germination rates and growth.

H₃. Because seed-set and mass may fluctuate by environmental conditions (Jordano 1993; Huyghe et al. 2002; Prasad et al. 2006), I predicted that the number of seeds produced, and the size of these seeds, would vary by site, with the irrigated garden site producing larger, and greater amounts of, seeds.

H₄. Due to the isolation of these populations, I hypothesized that populations of wiliwili would be genetically distinct, from island to island, and possibly from population to population.

H₅. Due to the aforementioned pressures on the regeneration of this species, I hypothesized that population structure is skewed toward mature individuals, with very little recruitment.

H₆. I hypothesized that populations with high levels of non-native grasses and non-native insect predators (the African bruchid beetle and the *Erythrina* Gall Wasp) would show the lowest recruitment.

Chapter two presents my pollination biology study. Chapter three focuses on wiliwili population genetics. Chapter four discusses the structure of wiliwili populations. Finally, in Chapter five, I present my conclusions with conservation implications.

CHAPTER 2. POLLINATION BIOLOGY OF *ERYTHRINA SANDWICENSIS*

Abstract

Dryland forest ecosystems have all but disappeared across the globe, and the trend is consistent in Hawai‘i. One of the most iconic species in Hawaiian dry forests is the endemic Hawaiian Coral Tree, *Erythrina sandwicensis*, or wiliwili. Little was known about the reproductive ecology of this culturally important species. This study aimed to identify the breeding system and types of visitors to wiliwili flowers. Two sites were chosen to conduct hand-pollination trials and to observe visitors, one garden and one wild site on O‘ahu, plus an additional site on Hawai‘i Island to observe floral visitors. For the breeding system work, I compared fruit set, seed set, seed germination, seedling growth rate, and seedling size across four pollination treatments: open control, autogamy, geitonogamy, and xenogamy. I found that wiliwili is visited by a novel suite of non-native visitors. All treatments produced seeds, but the xenogamous (cross) treatment produced significantly more fruit and seeds than the control or other treatments. Seedlings produced from cross-pollinated treatments were also taller and had wider basal-diameters after twenty-eight days of growth. These results indicate that wiliwili employs a mixed-mating system, and that these trees are pollen limited. I recommend that larger populations of wiliwili be established to promote cross-pollination.

Introduction

The flowering plants of Hawai‘i have experienced a great amount of speciation with about ninety percent of all taxa being endemic, or, found nowhere else in the world (Sakai et al. 2002; Ziegler 2002). Endemic species are far more likely to be at risk than indigenous species (Sakai et al. 2002). This is because of the archipelago’s geologic history and isolation from mainland continents, where species that have evolved here have done so in the absence of pressures, such as parasites and herbivory, that indigenous species have evolved with (Cox and Elmqvist 2000; Ziegler 2002). It is not surprising that Hawai‘i has “more endangered species per square mile than any other place on Earth” (Messing et al. 2007). More than half of native Hawaiian plant species (about sixty percent) are federally listed as endangered, and twenty-five

percent of these species occur in dry lowland, or scrubland ecosystems (Bruegmann 1996; Sakai et al. 2002).

Tropical dry forests are among the most imperiled ecosystems in the world. In Mesoamerica, less than two percent of these ecosystems are considered intact and worth preservation (Janzen 1988; Miles et al. 2006). A global assessment on the status of tropical dry forests by Miles et al. (2006) revealed that only a small portion of these ecosystems are designated as protected. In Hawai‘i, the pattern of loss is consistent with the global trend, with estimates of less than ten percent of these habitats still in existence (Merhoffer 1996, Sakai et al. 2002). However, when these estimates are broken down by island, subtler and more concerning patterns emerge. Sakai et al. (2002) report that more than ninety-nine percent of lowland dry forest and shrubland have been completely degraded on the islands of O‘ahu, Moloka‘i, and Lana‘i, with Maui having lost more than ninety-eight percent. In comparison, the Big Island of Hawai‘i has retained about seventeen percent of their dry forest habitat (Sakai et al. 2002), which skews the state’s estimated total area of remaining dry forest. Over the last two hundred years, a number of pressures have resulted in the destruction, fragmentation and degradation of these ecosystems including: land development, fire, the introduction of non-native plant, animal, and insect species, and the loss of mutualisms including pollinators and dispersers (Bruegmann 1996; Cabin et al. 2000; Cox and Elmqvist 2000; Messing et al. 2007; Cordell et al. 2008). It is estimated that twenty percent of species with dry, lowland distributions in Hawai‘i have gone extinct (Sakai et al. 2002).

A study by Pau et al. (2009) found that forty-five percent of taxa in dry forests are at risk of becoming endangered. One of the most iconic dry forest species is the Hawaiian wiliwili, Hawaiian Coral Tree, or *Erythrina sandwicensis* (O. Degener) (Fabaceae). Wiliwili is the only Hawaiian endemic species in a genus of one hundred fifteen species (Wagner et al. 1999).

Hawaiian wiliwili once dominated the landscape of lowland elevations, up to six hundred meters (Rock 1913; Medeiros et al. 2008). Its distribution is primarily restricted to the hottest, driest environments of the leeward sides on all of the main Hawaiian Islands (Rock 1913; Staples and Herbst 2005). This dry forest species was traditionally very important in Hawaiian culture

(Rock 1913; Medeiros et al. 1998; Staples and Herbst 2005). As culturally significant and abundant as this tree was, it is much less common today than in the past (B. P. Koebele 2016, personal communication, 23 December). In the context of imminent climate change, wiliwili is listed as one of seven Hawaiian dry forest taxa that is extremely at risk of endangerment (Pau et al. 2009). Today, surveys of population structure across the islands of O‘ahu and Hawai‘i show very little regeneration (Chapter 4).

Pollination ecology studies can yield significant insight on potential regeneration bottlenecks for species at risk, especially if native pollinators are thought to have declined or are no longer present (Kearns and Inouye 1993; Gardener and Daehler 2006). Although there is body of literature on the pollination ecology of other species in the genus *Erythrina*, there has been very little research conducted with wiliwili. Pollinators of the genus range from hummingbirds and passerine birds, to bees and, possibly, other insects and even bats, but the genus is thought to be bird-adapted (Baker and Baker 1983; Neill 1988; Bruneau 1997; Etcheverry et al. 2005, 2012, Galetto et al. 2014). Because of this early adaptation in its evolutionary history, the genus is thought to be largely xenogamous, or self-incompatible (Etcheverry et al. 2005; Etcheverry et al. 2012). Species that are highly xenogamous often experience limitations in reproductive success due to constrained pollen availability, and trials have shown that higher fecundity results from artificially outcrossed pollination (Burd 1994). One species (*E. crista-galli*) will set seed in autogamous (selfing) experiments successfully (Galetto et al. 2014), although the germination rates of these seeds were low (Etcheverry et al. 2005). Studies have shown that, even in partially self-compatible species, the probability of higher fruit and seed set and the chance that resulting seeds will have more mass, is greater with the application of outcrossed pollen (Jordano 1993; Rojas-Sandoval and Meléndez-Ackerman 2009).

Birds are known to be attracted to red or orange flowers that produce nectar (Gurevitch et al. 2006), like those of *Erythrina* species. The flowers of wiliwili, however, vary in color from red-orange to pale yellow and even light green. Because corolla color can affect the guild of floral visitors (Gurevitch et al. 2006), it is conceivable that the flowers of wiliwili are not exclusively bird-pollinated, but may be host to a new suite of visitors. A non-native, yellow-faced bee species (*Hylaeus strenuus*) has been observed visiting flowers (K. N. Magnacca 2017,

personal communication, 2 February) and vertebrate visitors to the flowers of wiliwili include non-native birds such as red-vented bulbuls (*Pycnonotus cafer*), common mynas (*Acridotheres tristis*), and Japanese white-eyes (*Zosterops japonicus*) (Staples and Herbst 2005).

In an effort to collect data that produces useful, informative results for conservation management of wiliwili, I addressed the following questions:

1. What species are the current flower visitors of wiliwili?
2. What is the effect of different pollination treatments on the number, size, and viability of wiliwili seeds and seedlings?
3. Do the number and size of seeds produced vary across sites?

Due to the loss of possible native pollinators, I hypothesized that wiliwili is visited by a novel guild of potential pollinators to the genus.

Because of the tendency for species in this genus to be self-incompatible, I hypothesized that seeds produced from spatially distant outcrossing treatments would have higher seed and fruit set, greater seed mass, and higher germination rates and growth.

Because seed-set and mass may fluctuate by environmental conditions (Jordano 1993; Huyghe et al. 2002; Prasad et al. 2006), I predicted that the number of seeds produced, and the size of these seeds, would vary by site, with the irrigated garden site producing larger, and greater amounts of, seeds.

Methods

Study species

Erythrina sandwicensis is found on all eight of the Main Hawaiian Islands: Ni`ihau, Kaua`i, O`ahu, Moloka`i, Lana`i, Maui, Kaho`olawe, and Hawai`i up to 600 m elevation (Rock 1913, Wagner et al. 1999). Wiliwili is a tall tree, reaching heights up to 15 m, with reddish, papery bark and narrow cracks that run the length of the trunk. The trunk and the branches are sporadically armed with conical prickles. This species is a summer deciduous tree, losing its leaves (usually) while flowering. Inflorescences are 10-15 cm long, with a horizontal axis and flowers only occurring along the distal half on pedicels 3-10 mm long. The corolla varies in color, from red-orange (usually) to yellow, white, and even pale green. These colors of flowers

may be present on trees in one population, and sometimes on the same tree. The flowers are papilionoid and form a reflexed, broadly elliptic claw (4-5 cm long), with the wing petals being elliptic and somewhat hooded (1.5-2 cm long). The keel petals are distinct, elliptic, hooded, and a bit shorter than the wings. Stamens are monodelphous (4-5 cm long), in the typical 9 + 1 arrangement; the adaxial stamen distinct in the upper portion. The ovary is stipitate, 2-2.5 cm long, and stellate pubescent. The style is filiform, 2-2.5 cm long, and crowned with a capitate stigma (Fig. 2.1). The fruit is a leguminous, somewhat woody, pendent pod with a papery endocarp, containing 1-3 seeds. These seeds are red to yellowish orange, about 1.5 cm long and 1 cm wide, and remain fixed to pods long after they've matured (Wagner et al. 1999)

Study sites

This research was conducted at two sites on the island of O'ahu, at the Mākua Kea'au Forest Reserve on the western (leeward) side, and Koko Crater Botanical Garden (City and County of Honolulu Conservation District) . These two sites, one garden and one wild, were chosen to represent variation in resource availability, particularly water, that might influence seed set, size, and vigor. The garden site is irrigated while the wild site is not. Total average annual rainfall for the garden and wild site were approximately the same (30-40 inches/year) (Guide of US 2018). The trees in the garden site are almost all naturally occurring (~83%) (N. Hoffman, 2018, personal communication). I also carried out pollinator observations on the leeward side of the Big Island (Hawai'i Island) at the Waikoloa Dry Forest Preserve. Pollination experiments and pollinator observations were carried out from June to October 2017. Nectar concentration measurements were conducted from July to August 2017.

Experimental design

A pilot study was conducted to determine the longevity of the flowers, their ontogeny, the timing of anthesis and stigma receptivity, and fruit maturation rate. Flower longevity and their ontogeny were determined by closely monitoring the flowers for a one-week period. Timing of anthesis and stigma receptivity were assessed by bagging flowers on one day, then reopening the following day to check for the presence of pollen on anthers and the adhesiveness of the stigma throughout that second day. The pilot study was also important in deciding the type of material

to use when bagging experimental inflorescences, the method of identifying treated flowers, the number of flowers to treat, and whether the remaining untreated flowers could remain on the same inflorescence as treated flowers. Based on the results of this pilot study, and other research on resource allocation (Medrano et al. 2000), it was determined that untreated, distal/late-opening flowers would be trimmed to avoid resource competition and accidental self-pollination within the bagged, treated flowers.

To determine what animals are visiting the flowers of the wiliwili tree, I watched randomly chosen sets of inflorescences (in 15 minute increments) at Koko Crater Botanical Garden (2 hours), Mākua Kea‘au (2 hours), and Waikoloa Dry Forest (4.5 hours), for a total of 8.5 hours. To account for any environmental variability, I recorded the time of day, light level (lux when available, or generic—Full sun, partial, etc.), wind speed (m/s), relative humidity (RH) (%), and temperature (degrees Celsius) every 15 minutes (Kearns and Inouye 1993). When recording, I noted the identity of the animal, and if it contacted reproductive parts of the flower.

To determine the breeding system of wiliwili, five pollination treatments were carried out on seven trees at Koko Crater Botanical Garden and eight trees in the Mākua Kea‘au population, for a total of 15 trees. I performed the following treatments: a) natural pollination (uncut), in which flowers were left open to natural pollination and the unmarked flowers of the inflorescence were not removed, b) natural pollination (cut), in which flowers were not manipulated but the remaining unmarked flowers were removed, c) autogamous self-pollination, in which flower buds were bagged throughout their development, d) geitonogamous self-pollination, in which bagged flowers were emasculated and hand-fertilized with pollen from a flower on the same tree, and e) xenogamous cross-pollination, in which bagged, emasculated flowers were hand-fertilized with pollen from a donor tree at least 20 m away. Two control treatments (the first two treatments mentioned above) were performed to account for the possible effects of resource allocation on fruit and seed set (Medrano et al. 2000). Every tree received every treatment, but some trees had two or three replications of treatments due to the accessibility of inflorescences. A total of 20 replicates per treatment were carried out.

Inflorescences were bagged to exclude possible pollinators using white, organza mesh bags (22 in. X 25.5 in.). Wire hangers were manipulated in a way such that each side ran perpendicular to the other, forming a cone-like structure to prevent potential damage to the flowers from rubbing against the bag, as well as preventing accidental self-pollination. These were inserted around the inflorescence, and tied to the corresponding branch, before the bag was pulled over the treated flowers. Electrical wire, varying in colors, were used to indicate the treatment type for each flower.

The number of flowers treated, the resulting fruit and seed set from each treatment, and the mass of these seeds were recorded using a Mettler AE100 analytical balance. After obtaining the mass, each seed coat was clipped using gardening sheers, and then each placed in an individual two-inch pot containing a mix of Sunshine® Mix #4 and perlite for germination. This germination experiment was conducted in the Pope Environmental Laboratory glasshouse at the University of Hawai‘i at Mānoa. Pots were watered as frequently as needed to prevent desiccation and observed for germination daily. A seed was counted as having germinated when radicle emergence was observed. The germination experiment continued for 28 days, though the germinated seeds did so within 14 days or did not germinate. Once per week, for 28 days, the resulting seedlings were measured for height and diameter to test for viability. Height was taken by measuring the distance from the soil line to the apical meristem, and diameter was measured just below the cotyledons. After 20 and 37 days, the total leaf area was recorded using CID Bio-Science’s CI-203 Handheld Laser Leaf Area Meter.

The nectar concentration experiment was conducted using the protocols of Baker and Baker (1983). Sucrose concentration analysis of the nectar was conducted using a hand-held refractometer and microcapillary tubes. The refractometer measures sucrose concentration as a percentage. Data were collected on 27 bagged and open flowers at different times throughout the day, and on separate days. These flowers were roughly at the same stage in their ontogeny, and the time and day were recorded. Nectar was extracted without removing the flowers from the tree and done in a way to avoid damaging the nectaries.

Statistical analyses

To identify the breeding system, I tested the effect of pollination treatment and site on the number of seeds set per flower pollinated, and the number of seeds set per fruit, using a generalized linear mixed model (GLMM) with a zero-inflated, Poisson distribution, using the glmmADMB package (Fournier et al. 2012; Skaug et al. 2013). The effects of pollination treatment and site on number of fruit set per flower pollinated was tested using Penalized Quasi-Likelihood, with a quasipoisson distribution in the MASS package (Venables and Ripley 2002). To test the effect of pollination treatment and site on seed mass, days to germination, seedling growth rate, and seedling size (height, leaf area, and diameter), I fit linear mixed effects models with normal distributions utilizing the nlme package (Pinheiro et al. 2018). To test the effect of the pollination treatment and site on the probability of germination, I fit a GLMM with a binomial distribution in the glmmADMB package (Fournier et al. 2012; Skaug et al. 2013). For all models, individual tree was considered a random effect and interactions between the covariables were included. R-squared values were calculated with the dredge function, and conditional pseudo-R-squared values were obtained using the r.squared GLMM function, both in the MuMIn package (Bartoń 2013). I used the model selection procedure based on the conservative Akaike's Information Criterion (AIC), also known as AIC model-reduction, to determine the best-fit model when considering all covariates (Akaike 1974). All analyses were conducted in R Studio, version 3.4.3.

There was no significant difference between the two controls of cut and uncut inflorescences ($P = >0.05$) in any of the tested models. Therefore, contrasts were carried out comparing treatments with the trimmed inflorescence control. This is the control that is reported in the results.

Results

The pilot study revealed that wiliwili demonstrates acropetalous inflorescence development, where flowers progress from the base toward the apex, having both proximal/early-opening flowers and distal/late-opening flowers. Flower buds push through the calyx, resembling a bird's beak or claw, and open within one or two days from emergence. Open

flowers remain so for three to four days before senescence, losing banner petals first and keel petals second. Anther dehiscence and stigma receptivity seem to occur simultaneously, suggesting the flowers are homogamous (where both stigma receptivity and anther dehiscence occur synchronistically). Anthesis seemed to occur early in the morning, perhaps while in the absence of light, and stigmas were also receptive in the morning (0600 - 1100 AM). Stigma receptivity lasted throughout the day and decreased as evening approached and flowers began to senesce. Anthers and stigmas are persistent, often remaining in the infructescence. Fruit pods develop within two days from flower senescence. Ripe pods, however, take six to eight weeks to fully mature and begin to dehisce. Once fully mature, pods dehisce partially or completely via one suture that runs the length of the fruit.

Nectar

Wiliwili produces copious amounts of nectar, sometimes exuding from the flower with the slightest contact. Sucrose concentration in the nectar of wiliwili flowers ranged from 10% to 18%, with a mean concentration of 14.2% ($\pm 1.9\%$). This concentration became greater toward the evening (Table 2.1).

Table 2.1. Nectar sucrose concentration (%) by time of day

Time of day	Mean % concentration \pm SD
0900-1059	12.8 \pm 1.5
1100-1259	13.8 \pm 1.8
1300-1459	15.9 \pm 0.9

Floral visitors observed on wiliwili

Floral visitors to the flowers of wiliwili were all non-native. Invertebrate species included: Western honey bees (*Apis mellifera*), carpenter bees (*Xylocopa sonorina*), small carpenter bees (*Ceratina smaragdula*), yellow-faced bees (*Hylaeus strenuous*), and vespid (or paper) wasps (*Polistes* sp.) (Fig. 2.2). Of these, only carpenter bees were seen to contact both reproductive parts of the flower within one observational period. Yellow-faced bees and honey bees were observed to contact anthers but not stigmatic surfaces. Paper wasps never contacted any reproductive parts. Vertebrate visitors included: Common myna birds (*Acridotheres tristis*),

red-vented bulbuls (*Pycnonotus cafer*), Japanese white-eyes (*Zosterops japonicus*), and red-crested cardinals (*Paroaria coronata*) (Fig. 2.3). In the Waikoloa Dry Forest Preserve, a female house finch (*Haemorhous mexicanus*) and a saffron finch (*Sicalis flaveola*) were also seen visiting the flowers, as well as a small carpenter bee (*Ceratina smaragdula*). Contact with reproductive parts was undeniably observed in common myna bird foraging, but not observed with absolute certainty in other bird species.

With anthesis occurring early in the morning, the first visitors to the flowers were insects. Western honey bees and non-native yellow-faced bees collected pollen furiously in these morning hours, creating a high-pitched frequency that sounded all through the forest. As the day progressed, foraging behavior changed for the honey bees, switching from pollen collectors to nectar robbers. Honey bees systematically avoided stigma contact with every visit, climbing filaments only, or entering from the base of the flower and maneuvering keel petals about until locating the reward. Yellow-faced bees appeared focused on pollen collection, leaving contacted anthers completely devoid of any male gametes. My field assistant and I entertained the idea of functional gynodioecy evolving in this species, until we bagged the flowers overnight. To our surprise, we found bagged flowers (excluded from any visitors) contained generous amounts of pollen. Paper wasps collected nectar only, and were seen as early as 0900 AM, but never contacted any reproductive organs. Wasps were observed perching on banner petals, collecting the abundantly flowing sugar-water that dripped from the nectaries down the tissue. Carpenter bees were seen foraging for nectar as well as pollen, contacting both anther and stigmatic surfaces. Insects were not observed in rainy weather, which was expected and consistent with the research conducted by Vicens and Bosch (2000).

At midday, the most consistent visitors witnessed (across all sites) in the wiliwili trees were myna birds. They enter the flower from the rear and obtain nectar. However, their large bodies knock anthers and stigmas about while foraging from one flower to the next, one inflorescence to the next, and one tree to the next. In doing so, common mynas may be providing a service to these trees. The same could be said for the red-vented bulbuls and red-crested cardinals, though these species were not observed as often, not definitively contacting anthers and stigmas concurrently, and only in the O‘ahu sites. In contrast, Japanese white-eyes were the

only vertebrate visitor able to avoid contact with reproductive parts all together, owing to their smaller body size. White-eyes were observed clutching banner petals with their feet (and damaging them), entering flowers from the back side to collect the sugary reward—nectar robbing. White-eyes (and possibly the finches) were able to nectar rob without contacting reproductive parts, but larger birds (common mynas and red-vented bulbuls) were not able to avoid these parts while exclusively aimed at nectar acquisition.

Effects of pollination treatment and site

The cross-pollination treatment produced significantly more fruit (Fig 2.4, $P = 0.006$, Appendix Table A1) and more seeds (Fig 2.4, $P < 0.001$, Appendix Table A2) per flower pollinated, than the control, autogamous, or geitonogamous treatments. A total of 30 fruits were produced from 110 flowers (27%) treated with pollen from a donor tree more than 20 m away from a recipient tree versus 9/139 (6%) from the control (left open to natural pollination with the unopened flowers trimmed away). The cross-pollination treatment produced 57 seeds from 110 flowers (52%), versus 9/139 (6%) from control, 5/113 (4%) from the autogamous treatments, and 10/107 (9%) from the geitonogamous treatments (Fig. 2.5). The maximum cross-pollinated seed set was 5 in one fruit at Mākua Kea‘au. There was no significant difference in seed set per fruit among treatments or sites (Fig. 2.6, Appendix Table A3).

Pollination treatment had no significant effect on seed mass, but seed mass differed between sites, with Mākua Kea‘au seeds being significantly lighter than those from Koko Crater ($P = 0.006$, Fig. 2.7, Appendix Table A4).

Pollination treatment also had no significant effect on the probability of germination. However, seeds from Mākua Kea‘au were significantly less likely to germinate (48% germination) than those from Koko Crater (86% germination; $P = 0.007$, Fig. 2.8, Appendix Table A5). Seed mass was a significant predictor of the probability of germination ($P = < 0.001$, Appendix Table A6).

Of the seeds that germinated, the mean number of days to germination was 6.48 ± 1.94 days and did not differ significantly across pollination treatments or sites (Table 2.2, Appendix Table A7). Seedling growth rate (growth per day) did not differ significantly among

pollination treatments or sites (Fig. 2.9, Appendix Table A8). Upon further analysis, seed mass was determined a significant predictor of, and positively correlated with, growth rate ($P = <0.001$, Appendix Table A9), which was significantly higher in seedlings from Mākua Kea‘au when controlling for the mass of the seed ($P = 0.045$, Appendix Table A9). Seeds from Mākua Kea‘au had lower seed mass overall, there is not difference in growth. However, when looking at seeds with the same mass (small seeds in this case), they grew faster.

The final size of the seedlings after one month, regardless of the number of days it had been since they had germinated, varied significantly across pollination treatment and site, depending on the measure used. Seedlings produced by crosses were significantly taller than the control seedlings and those produced from autogamous or geitonogamous treatments ($P = 0.038$, Fig. 2.10, Appendix Table A10). Site had no significant effect on seedling height. Neither pollination treatment nor site had a significant effect on total leaf area (Table 2.2, Appendix Table A11). The basal diameter of seedlings produced by the cross treatment were significantly greater than the open-pollination control ($P = 0.002$), and other treatments, but site had no effect (Fig. 2.11, Appendix Table A12). Seed mass was a significant predictor of seedling height (Fig. 2.12, Appendix Table A13).

Table 2.2. Germination day and leaf area experiments (by treatment)

Experiment	Control	Auto	Geit	Cross	Mean \pm SD
Days to germinate	8	7.5	6.14	6.08	6.48 \pm 1.94
Leaf area (cm²)	77.06	57.07	56.79	101.49	61.15 \pm 54.54

Discussion

Nectar

Flowers of *Erythrina sandwicensis* produce a dilute nectar (mean of 14%) (Johnson and Nicolson 2008), and in what appeared to be very high amounts relative to other Hawaiian taxa. As the time of day grew closer to evening, however, concentration was greater possibly due to evaporation in the sun. Since the flowers of wiliwili are open, however, the low concentration of the nectar could be due to the high osmolality of the sugar drawing water from the plant to the flowers (Nicolson 2002). These simple nectar measurements, with a low sucrose concentration,

support a generalized, ornithophilous (or bird-adapted) pollination syndrome (Johnson and Nicolson 2008). This is consistent with the types of visitors witnessed foraging in the trees, and with all other species in the genus (Faegri and Van der Pijl 1979, Bruneau 1997).

Floral visitors

Japanese white-eyes did not seem to be an effective pollinator of the wiliwili trees. They did more damage to the flowers than service while obtaining nectar. Another study showed that white eyes were exclusively nectar robbing from the flowers of *Cyanea superba* and *Delissea waianaeensis* (Pender 2013). This was due to a case of mismatch morphology between the bird and the flower, and I postulate this is the same case for the wiliwili flower, though direct floral and bill morphology comparisons were never conducted.

Pollination services may be effectively provided with non-native species such as the common myna. Another study showed that non-native visitors can be legitimate pollinators of endemic Hawaiian plants (Aslan et al. 2014). Common mynas forage across the entire tree, before flying off to a new tree, searching for nectar and/or water. Their bodies definitely contacted both anthers and stigmatic surfaces in this process. In the absence of a possible native pollinator, these birds have established a novel mutualism with the wiliwili tree, at least in the three sites I observed.

Carpenter bees also seem to have established a mutualism with the flowers of wiliwili. Other insect visitors (paper wasps, yellow-faced bees, and honey bees) did not contact both reproductive parts, but carpenter bees are much larger than these insects. Their bodies are almost as large as the wiliwili flowers, making it difficult for them to avoid stigmas and anthers while foraging about.

One limitation of the study was access to the inflorescences. Trees reached heights up to fifteen meters, and binoculars can only yield so much observation. A better way to access the canopy is recommended for further studies.

Breeding system

Fruit set and seed set per flower was greater under cross-pollinated conditions than under the autogamous and geitonogamous treatments (Fig. 2.13). This suggests that wiliwili is a primarily outcrossing species, but not entirely so. Some seeds were produced from geitonogamous and (very rarely) autogamous treatments, suggesting that wiliwili employs a mixed-mating system. Self-pollination may eventually lead to a loss of vigor from excessive inbreeding (Campbell 2011, Etcheverry et al. 2012). This implies there must be some internal mechanism that only allows pollen from the same tree to flow through the pollen tube as a back-up system for reproductive assurance, which is common in island species (Robertson et al. 2011). Whether or not these “selfed” seeds survive to adulthood and are capable of producing seeds themselves has yet to be tested. Results from the pollination treatments indicate that this species has a similar mating system to most congeners, and a relative in the Eastern Andes, *E. dominguezii*, which Etcheverry et al. (2012) describe as “incomplete self-incompatibility.” It should be noted that autogamous treatments could not be fully substantiated as such, due to the bagging of the entire inflorescence with several open flowers. It is possible that pollen from an open flower within the bag found its way to the stigma of another flower, or the same flower, confounding the spontaneous pollination results. Therefore, it is recommended that future studies manipulate the bagged inflorescences in such a way to prevent this occurrence or emasculate the flowers by trimming the anthers.

Flowers that were treated with cross-pollen from a donor tree several meters away produced more seeds than the open control. This suggests that wiliwili trees, at both the garden and wild sites, are pollen limited. This is often the case with species that are self-incompatible (Burd 1994). Because observed floral visitors are not native to Hawai‘i, these trees have not had the opportunity to coevolve with them. Naturally pollinated flowers did set seed in some cases, but are pollen limited suggesting that these non-native visitors may not be so effective as pollinators.

When holding the amount of fruit constant, the cross treatment did not yield more seeds. Logically, the cross produces more seeds because it produces more fruit. Another study (on a

columnar cactus) showed that cross-pollinated seeds, in a mixed mating system, germinated 1.5 times more often than did seeds from natural or self-pollination treatments. Consequently, these “crossed” seedlings had higher values of “multiplicative fitness” (Rojas-Sandoval and Meléndez-Ackerman 2009). This was not the case with wiliwili, where pollination treatment had no significant effect on the probability of germination. However, the size of a wiliwili seedling (height and diameter) after 28 days was significantly larger (an average of 3.68 cm taller and 1.54 mm thicker) for cross-pollination treatment than for the other treatments. This suggests that the overall vigor of the cross-seedlings was significantly higher as well. The lack of difference across pollination treatments in leaf area may have been confounded by insects that ate the true leaves of five plants before measuring could take place, and stem rot that occurred in six seedlings (both cross and self-treatments); killing the plant before the leaf area could be taken.

Regardless of treatment, more seeds were produced from the Koko Crater Botanical Garden site, and the mass of these seeds was greater than the wild site (Mākua Kea‘au). This could be due to the availability of resources (mainly irrigated water) at the garden site, or due to the population’s genetic phenotype. A seed with greater mass has a higher probability of germinating, and the mass of the seed determined the height of the seedling. This is consistent with the results of Baraloto et al. (2005) where larger seeded species produced larger seedlings that were more likely to survive in eight species tested from the neotropics. Overall, seed mass was lighter at Mākua Kea‘au, and these seeds germinated less even when mass was held constant, so something unknown is occurring. Rates of germination in this study (~73%) were consistent with Medeiros et al. (2008) where they found 68.5% of seeds germinated without bruchid beetle eggs or exit holes. In Chapter 4, I found that roughly 16.7% of seeds on the ground at Waikoloa Dry Forest were infested with this beetle’s larvae.

In the context of climate change, lighter seeds could be problematic for species residing in dry forest habitats. A study by Frazier and Giambelluca (2017) showed an average decrease of 1.78% in annual rainfall per decade since 1920 across the state. Some of the most “significant downward trends” (on O‘ahu) were seen in “mountainous regions” where dry season rainfall was decreasing drastically (Frazier and Giambelluca 2017). This is where wiliwili is found at Mākua Kea‘au. If mass is a significant predictor of seed germination, seedling size, and survivability,

and rainfall is steadily decreasing with climate change, wiliwili may be unable to mitigate these shifting conditions in regard to regeneration. Additionally, the drying trend will likely contribute to an increase in wildfires throughout the state (Trauernicht et al. 2015). Conservation implications associated with these results are discussed further in Chapter 5.

Fig. 2.1. Labeled photo of wiliwili floral morphology



Fig. 2.2. Invertebrate visitors to wiliwili flowers



Fig. 2.3. Avian visitors to wiliwili flowers



Fig. 2.4. Number of fruit set per flower as a function of pollination treatment and site (control = cut control [2], auto = autogamous, geit = geitonogamous, cross = outcross)

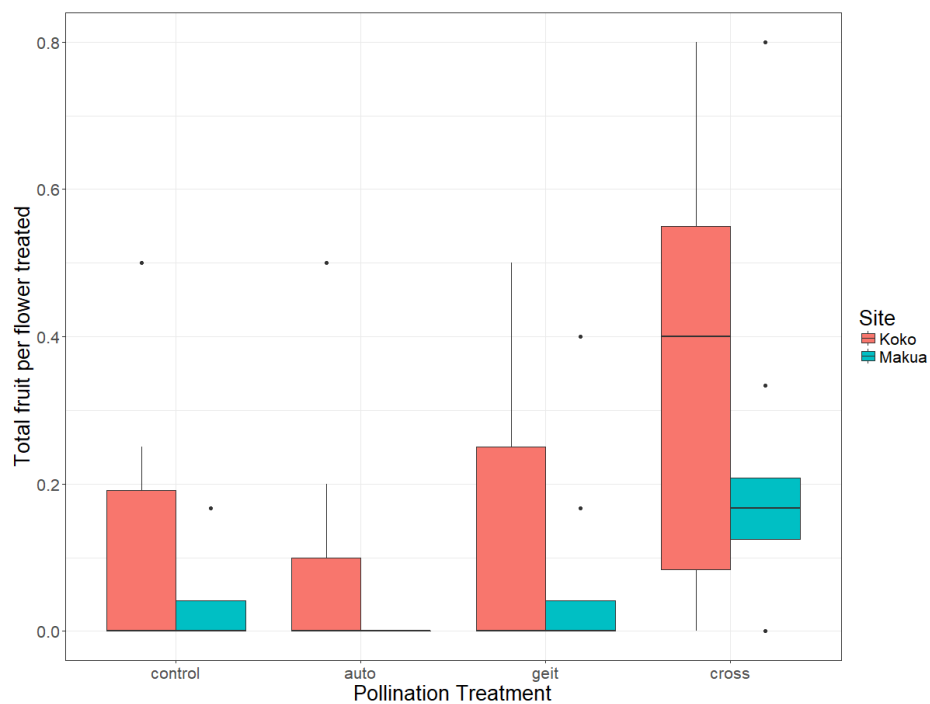


Fig. 2.5. Number of wiliwili seeds set per flower, as a function of pollination treatment and site (control = cut control [2], auto = autogamous, geit = geitonogamous, cross = outcross)

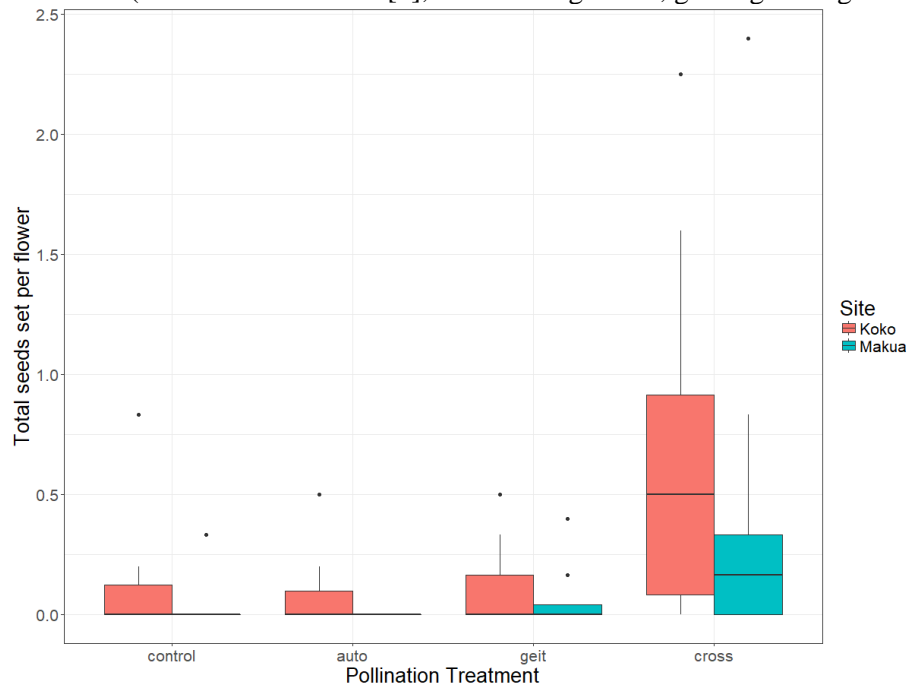


Fig. 2.6. Number of seeds set per fruit as a function of pollination treatment and site (control = cut control [2], auto = autogamous, geit = geitonogamous, cross = outcross)

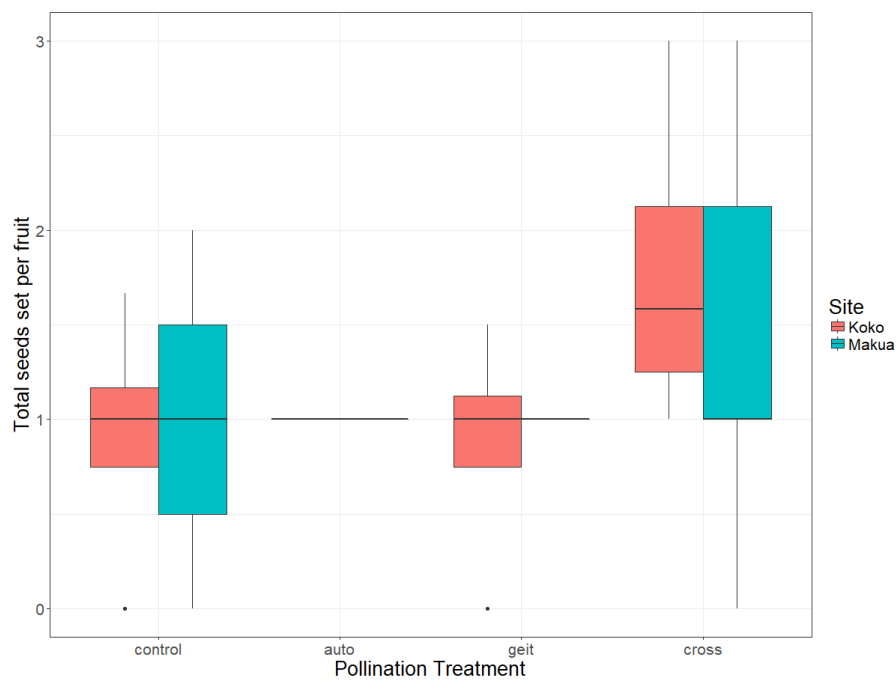


Fig. 2.7. Effect of pollination treatment and site on seed mass (control = cut control [2], auto = autogamous, geit = geitonogamous, cross = outcross)

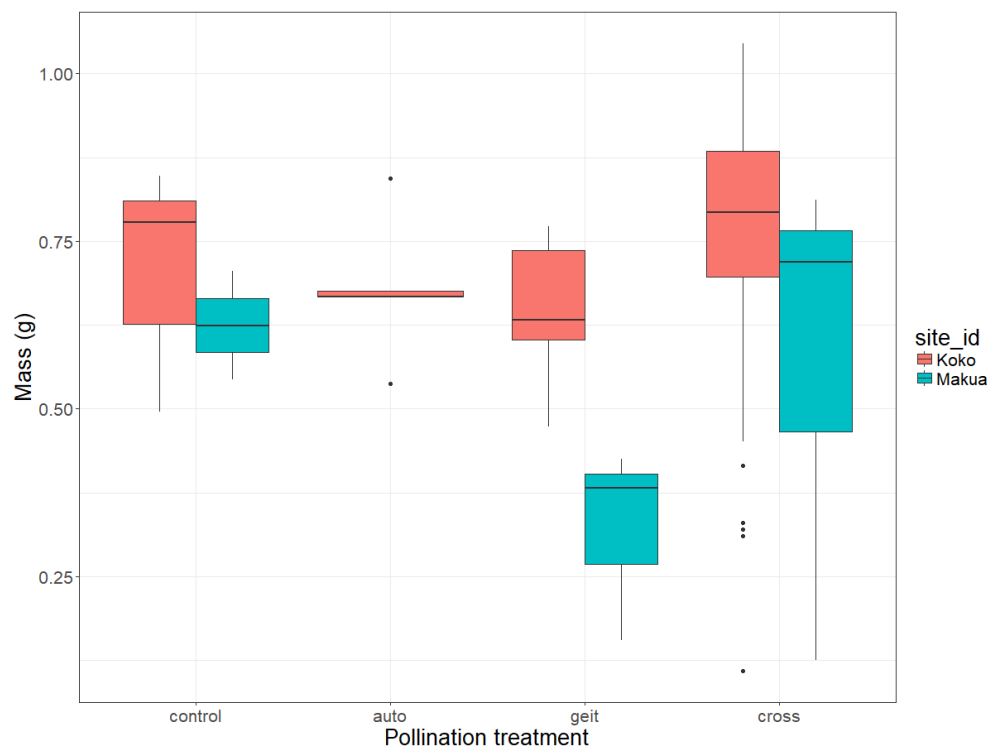


Fig. 2.8. Probability of germination as a function of pollination treatment and site (auto = autogamous, geit = geitonogamous, cross = outcross)

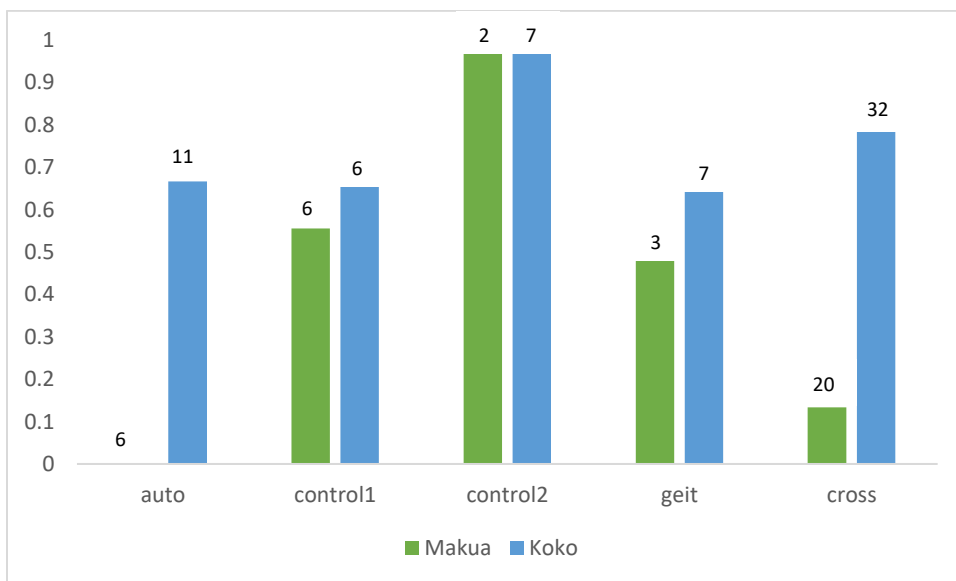


Fig. 2.9. Seedling growth rate (growth per day) as a function of pollination treatment and site (control = cut control [2], auto = autogamous, geit = geitonogamous, cross = outcross)

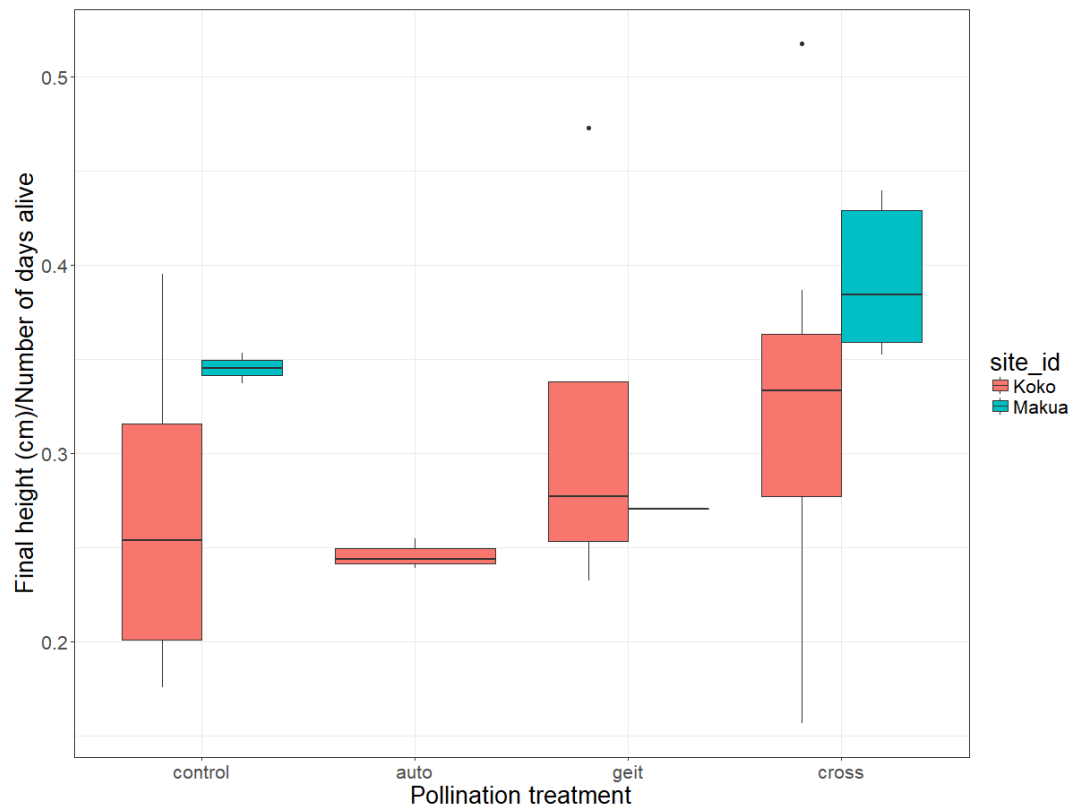


Fig. 2.10. Effect of pollination treatment and site on seedling height 28 days after planting (control = cut control [2], auto = autogamous, geit = geitonogamous, cross = outcross)

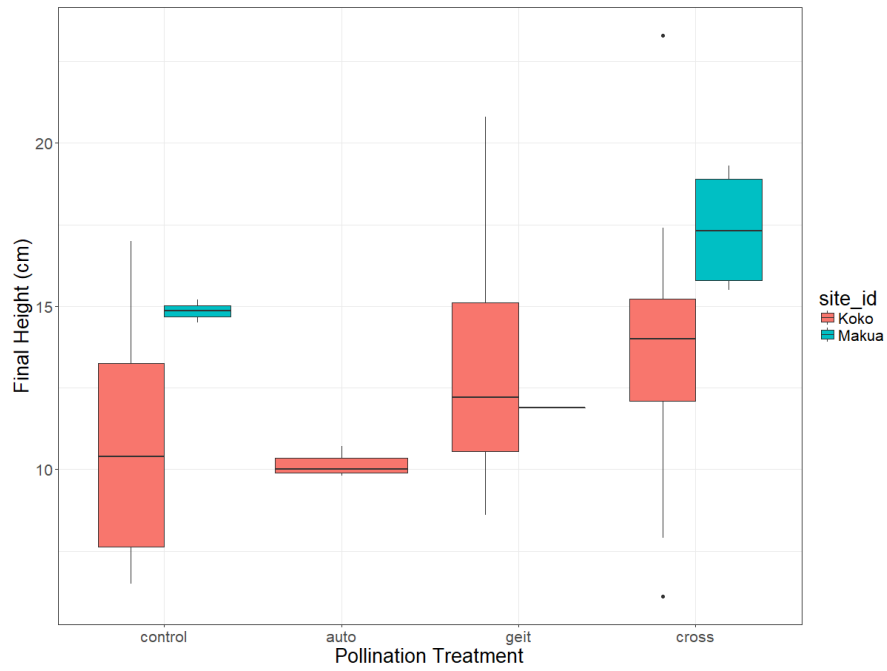


Fig. 2.11. Effect of pollination treatment and site on final seedling basal diameter 28 days after planting (control = cut control [2], auto = autogamous, geit = geitonogamous, cross = outcross)

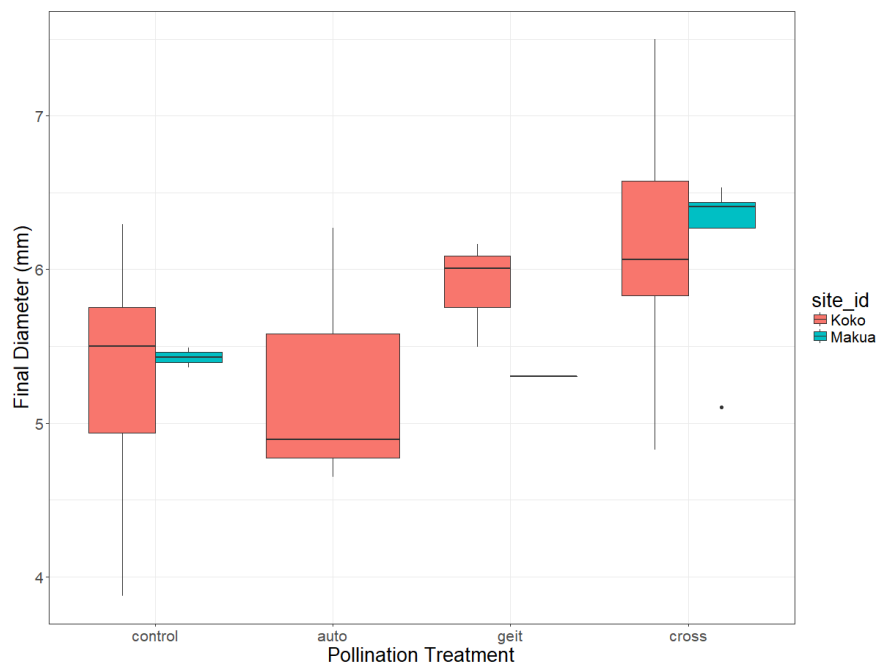


Fig. 2.12. Effect of seed mass on seedling height 28 days after planting

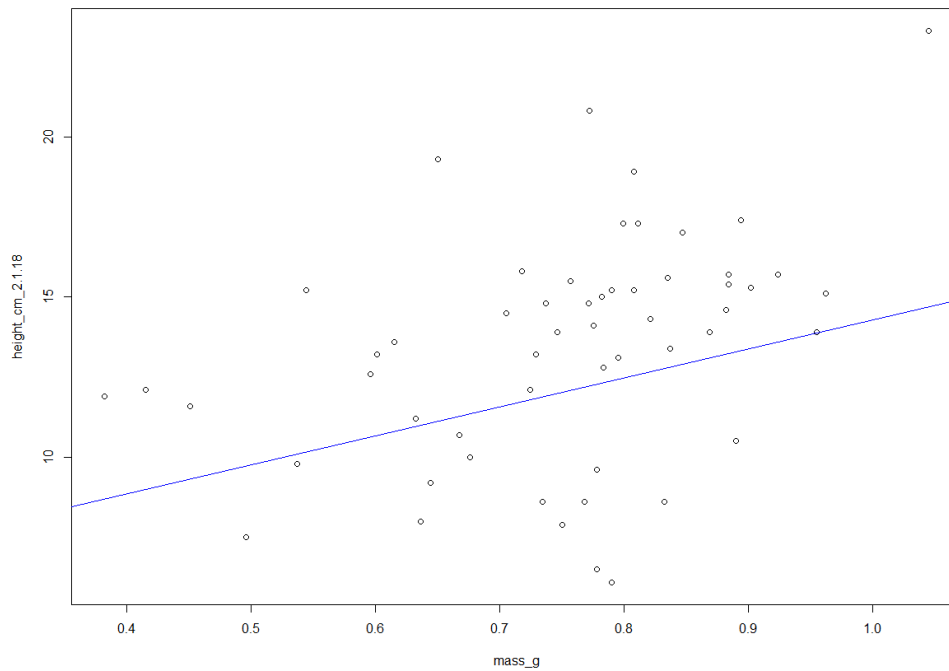


Fig. 2.13. Summary of breeding system results: Arrows pointed up or down indicate significant increase or decreases, respectively. “X” indicates that no significant difference was found. For site, arrows pointed down indicate that values were lower at Mākua Kea‘au than at Koko Crater.

	Seeds/flower	Fruit/flower	Seeds/fruit	Mass (seeds)	Prob. Germ.	Time to germ	Height	Leaf area	Diameter
Cross	↑	↑	×	×	×	×	↑	×	↑
Site	×	×	×	↓	↓	×	×	×	×
Mass	--	--	--	--	↑	×	↑	×	×

CHAPTER 3. POPULATION GENETICS OF HAWAIIAN WILIWILI

Abstract

Dryland forest ecosystems are globally endangered, and this is true of Hawaiian dry forests as well. An iconic species known from these systems is the endemic Hawaiian Coral Tree, *Erythrina sandwicensis*, or wiliwili. Wiliwili inhabits all eight of the Main Hawaiian Islands, but little was known about the relationships among these island populations. The goal of this study was to determine the genetic relationships and genetic identity of wiliwili populations. To do this, 71 samples were randomly chosen from 274 individuals from nineteen populations on seven islands (except Ni'ihau) for sequence-related amplified polymorphism analysis (SRAP). Twenty primer pairs were chosen for the analysis from 130 primer combinations (13 forward and 20 reverse). From the data collected, analysis in the form of a similarity matrix, principle coordinate and STRUCTURE analyses, the percentage of polymorphism, and expected heterozygosity were performed. I found wiliwili populations to be genetically distinct from one another, and form four genetic groups: Kaua'i, O'ahu, the islands of Maui, Moloka'i, and Lana'i, and Hawai'i and Kaho'olawe. These results have conservation implications. Future research should investigate characteristics that may distinguish these island populations further.

Introduction

Tropical dry forests are among the most imperiled ecosystems in the world. In Mesoamerica, less than two percent of these ecosystems are considered intact and worth preservation (Janzen 1988; Miles et al. 2006). A global assessment on the status of tropical dry forests by Miles et al. (2006) revealed that only a small portion of these ecosystems are designated as protected. In Hawai'i, the pattern of loss is consistent with the global trend, with estimates of less than ten percent of these habitats still in existence (Merhoff 1996, Sakai et al. 2002). However, when these estimates are broken down by island, subtler and more concerning patterns emerge. Sakai et al. (2002) report that more than ninety-nine percent of lowland dry forest and shrubland have been completely degraded on the islands of O'ahu, Moloka'i, and Lana'i, with Maui having lost more than ninety-eight percent. In comparison, the Big Island of Hawai'i has retained about seventeen percent of their dry forest habitat (Sakai et al. 2002), which

skews the state's estimated total area of remaining dry forest. Over the last two hundred years, a number of pressures have resulted in the destruction, fragmentation and degradation of these ecosystems including: land development, fire, the introduction of non-native plant, animal, and insect species, and the loss of mutualisms including pollinators and dispersers (Bruegmann 1996; Cabin et al. 2000; Cox and Elmqvist 2000; Messing et al. 2007; Cordell et al. 2008). It is estimated that twenty percent of species with dry, lowland distributions in Hawai'i have gone extinct (Sakai et al. 2002).

The flowering plants of Hawai'i have experienced a great amount of speciation with about ninety percent of all taxa being endemic, e.g., found nowhere else in the world (Sakai et al. 2002; Ziegler 2002). Endemic species are far more likely to be at risk than indigenous species (Sakai et al. 2002). This is because of the archipelago's geologic history and isolation from mainland continents, where species have evolved in Hawai'i in the absence of pressures, such as parasites and herbivory, that indigenous species have evolved with (Cox and Elmqvist 2000; Ziegler 2002). The Hawaiian dry forests are one of the most endangered habitats in the world with only one percent of intact habitat remaining throughout the island chain (Sakai et al. 2002).

Erythrina sandwicensis (Deg.), or Hawaiian wiliwili, is an endemic, dry forest species found from sea level to ca. 600 m elevation (Rock 1913). Where the iconic wiliwili once dominated coast lines and foothills, it is now considered to be one of seven Hawaiian dry forest taxa that is extremely at risk of endangerment (Pau et al. 2009). The host-specific *Erythrina* Gall Wasp, *Quadrastichus erythrinae*, was discovered on the island of O'ahu in April 2005 where it spread to adjacent islands in just four months (Bell et al. 2013). This pest causes substantial defoliation and, in the worst scenarios, tree mortality in just a few months (Kaufman et al. 2014). A 2012 state-wide census revealed that an estimated 30-35% percent of wiliwili trees had died due to the gall wasp invasion (Kaufman et al. 2014). Another introduced arthropod to the Hawaiian Islands that limits recruitment of wiliwili is the African bruchid beetle, *Specularius impressithorax*. In 2001, the beetle was observed and collected in Makaha Valley on O'ahu island. It took only two years for this seed-predating beetle to become established on every main Hawaiian island (Medeiros et al. 2008). A study by Medeiros et al. (2008) found that the bruchid beetle accounted for seventy-seven percent of mean seed crop loss in twelve wiliwili

populations, on six of the main islands, in the first three years since its introduction. This is because one single larva can reduce the germination rate of a seed in a pod by ninety-seven percent (Medeiros et al. 2008). The gall wasp and bruchid beetle together may have catastrophic impacts on reproduction in wiliwili populations (Gumovsky and Ramadan 2011). Though not currently listed as endangered, the lack of regeneration in wiliwili populations is concerning (Kaufman et al. 2014). I wondered if, perhaps, the answer to solving the population structure problem lied in the genetic identity of each island population. If wiliwili populations arrived in the islands following the progression rule, and are genetically similar, conservation efforts could be identically applied across the island chain.

Many endemic Hawaiian taxa have arrived in the islands via long-distance dispersal, and subsequently follow a progression rule; colonizing the oldest islands first before dispersing to newly formed islands (Raven 1974, Ziegler 2002). However, not all taxa follow this rule as is the case with the endemic Hawaiian genus *Hesperomannia* (Morden and Harbin 2013). It is currently unknown if the endemic Hawaiian wiliwili follows this rule or was dispersed in some other manner. The closest relative of wiliwili is believed to be *E. tahitensis* (Nad.), from Tahiti (St. John 1956). However, this is only suspected since *E. sandwicensis* was not included in a phylogenetic, biogeographic treatment of the genus by Anne Bruneau (1996). This study suggests that most island endemic *Erythrina* species are derived relative to the African *E. burtii* which could have floated across the ocean (Raven 1974) or been carried to the islands via avian browsers due to the bright red seed's resemblance to a berry (Neill 1988).

The flowers of wiliwili vary in an array of pastel colors, from creamy white and green to salmon-orange, with every color between (Medeiros et al. 1998; Staples and Herbst 2005) and were once called “tiger’s claw” by island new-comers due to their claw-like form (Rock 1913). The seeds of wiliwili vary in color as well, from bright red to yellow, and are valued in the jewelry industry today, just as they were in the past (Rock 1913; Medeiros et al. 1998; Staples and Herbst 2005). The swollen trunks of these trees are conspicuously colored yellow to orange, with prickles that protrude from the branches (Rock 1913; Staples and Herbst 2005).

Wiliwili is notably characterized by having thick armory, or prickles (Rock 1913; Wagner et al. 1999; Staples and Herbst 2005). Harold St. John (1956) noted the Tahitian congener was “prickly” but “mostly unarmed.” In my own personal observations, I noted the lack of prickles on trees in Kaua‘i populations and the abundance of armory on trees in Hawai‘i (Big Island) populations. Trees in O‘ahu populations tend to have less prickles, or bumps rather than prickles, on the trunk than trees from Big Island, but the same amount of prickles on branches (especially young branches). Interestingly, the two trees of *E. tahitensis* residing at the National Tropical Botanical Garden (NTBG) on Kaua‘i did not possess any prickles at all. If wiliwili dispersed to Hawai‘i from Tahiti, it is probable that the first colonizers would have less armament. This trait may have evolved as propagules dispersed and became established on the other islands in progression.

Understanding the population genetics among islands can help to elucidate a species biogeographic pattern (Morden and Harbin 2013). This is important in the realm of conservation to avoid misclassification, or to facilitate conservation of genetic diversity, of an apparently wide-spread species (Funk et al. 2002). This study aims to identify the pattern of wiliwili gene flow among the Hawaiian Islands and determine the genetic variation therein. Conservation resources can then be more aptly applied with the knowledge of this variation. Therefore, my research question was:

What is the genetic relationship among wiliwili populations?

Due to the isolation of these populations, I hypothesized that populations of wiliwili would be genetically distinct, from island to island, and from population to population.

Materials and Methods

Study species

Erythrina sandwicensis is found on all eight of the Main Hawaiian Islands: Ni‘ihau, Kaua‘i, O‘ahu, Moloka‘i, Lana‘i, Maui, Kaho‘olawe, and Hawai‘i up to 600 m elevation (Rock 1913, Wagner et al. 1999). Wiliwili is a tall tree, reaching heights up to 15 m, with reddish, papery bark and narrow cracks that run the length of the trunk. The trunk and the branches are

sporadically armed with conical prickles. This species is a summer deciduous tree, losing its leaves (usually) while flowering. Leaves are pinnately trifoliate, with the terminal leaflet being deltate, or triangular, and wider than it is long (4-10 cm long and 6-15 cm wide), and the lateral blades being ovate (4-8 cm long, 4-9 cm wide). The upper surface of leaflets is glabrous, while the lower surface is densely stellate-tomentose. Petioles of terminal leaflets are 8-15 cm long, while petiolules of lateral leaflets are about 5 mm long. Subulate stipules (~5 mm long) are present on the petioles when young, but deciduous. A pair of glandular stipellae subtend the terminal and lateral leaflets along the petiole. Inflorescences are 10-15 cm long, with a horizontal axis and flowers only occurring along the distal half on pedicels 3-10 mm long. The calyx is fusiform in bud and densely stellate tomentose with a brown, felty texture, has 5 apical knobs, and splits open along the dorsal side as the corolla tissue emerges. The corolla varies in color, from orange (usually) to yellow, white, and even pale green. These colors of flowers may be present on trees in one population, and sometimes on the same tree. The flowers are papilionoid and form a reflexed, broadly elliptic claw (4-5 cm long), with the wing petals being elliptic and somewhat hooded (1.5-2 cm long). The keel petals are distinct, elliptic, hooded, and a bit shorter than the wings. Stamens are monodelphous (4-5 cm long), in the typical 9 + 1 arrangement; the adaxial stamen distinct in the upper portion. The ovary is stipitate, 2-2.5 cm long, and stellate pubescent. The style is filiform, 2-2.5 cm long, and crowned with a capitate stigma. The fruit is a leguminous, somewhat woody, pendent pod with a papery endocarp, containing 1-3 seeds. These seeds are red to yellowish orange, about 1.5 cm long and 1 cm wide, and remain fixed to pods long after they've matured (Wagner et al. 1999)

Plant materials

A total of 274 individual plants were collected from nineteen locations across seven of the main Hawaiian Islands except Ni'ihau (collected by Maya LeGrande, Cliff Morden, and Ken Wood). Up to four individuals were selected from each collection site (71 total individuals) for the sequence-related amplified polymorphism (SRAP) study (Table 3.1).

Table 3.1. Collections of *Erythrina sandwicensis* used from each population for SRAP analysis.
n=number of individuals for the population used in SRAP; Hawaiian Plant DNA Library
(HPDL)=accession number in the Hawaiian Plant DNA Library (Morden et al. 1996).

Island	N	Population	n	HPDL	Voucher
Hawaii	16	Hualalai	4	2194-2196, 2200	<i>LeGrande</i> s.n.
		Holei Pali	3	2896-2898	<i>LeGrande 1078abc</i>
		Puuloa	2	2899-2900	<i>LeGrande 1078de</i>
		Waimea	4	3307-3310	<i>LeGrande 1120</i>
		Waikoloa	3	5101, 5109, 5121	<i>C. Morden 1992</i>
Lanai	4	Awehi	4	2317-2320	<i>LeGrande 1074</i>
Maui	8	Kanaio	4	2305-2308	<i>LeGrande 1072</i>
		Mokumana Gulch	4	2883-2886	<i>LeGrande</i> s.n.
Molokai	11	Kawela	4	2282-2285	<i>LeGrande 1052-1055</i>
		Hoolehua	3	2298-2300	<i>LeGrande 1069-1071</i>
		Kauhako Crater_MO	4	2851-2854	<i>LeGrande 1076</i>
Oahu	16	Koko Crater	4	2177, 2178, 2180, 2182	<i>LeGrande 1020, 1021, 1023, 2025</i>
		Makakilo	4	1657, 1659- 1660, 1665	<i>C. Morden 1548</i>
		Mokuleia	4	2115-2118	<i>LeGrande 1001-1004</i>
		Waialae Nui Gulch	4	2835-2838	<i>LeGrande 1075</i>
Kahoolawe	8	Kahoolawe	4	1588-1591	<i>LeGrande</i> s.n.
		Kahoolawe	4	3286-3289	<i>LeGrande 1081</i>
Kauai	8	Waimea	4	2131-2134	<i>C. Morden 1604</i>
		Kapahili Gulch	4	2867-2870	<i>K. Wood 8350</i>
Total	71				

DNA extraction

DNA was sampled and extracted from each individual's fresh leaves using CTAB method by Doyle and Doyle (1987) with some modifications (Morden et al. 1996). The concentration and quality of DNA were determined using Nano Drop Spectrophotometer (ND-1000, v 3.6.0, Thermo Scientific). All DNA samples were diluted to 10-15ng/μl and stored at -20°C until use.

SRAP amplification

Sequence-related amplified polymorphism (SRAP), a polymerase chain reaction (PCR)-based marker system (Li and Quiros 2001), was utilized to investigate genetic variation among wiliwili populations. Five individuals from different populations were used to screen 130 different primer combinations for this study from 13 forward and 20 reverse primers (Tables 3.2 and 3.3). Twenty primer pairs that produced the most clear and reproducible bands were selected for the study (Table 3.3).

Table 3.2. The forward and reverse SRAP primers used for this study, adapted from Budak et al. (2004) and Liao et al. (2016).

Name	Forward Primer	Name	Reverse Primer
Me1	TGA GTC CAA ACC GGA TA	Em1	GAC TGC GTA CGA ATT AAT
Me2	TGA GTC CAA ACC GGA GC	Em2	GAC TGC GTA CGA ATT TGC
Me3	TGA GTC CAA ACC GGA AT	Em3	GAC TGC GTA CGA ATT GAC
Me4	TGA GTC CAA ACC GGA CC	Em4	GAC TGC GTA CGA ATT TGA
Me11	TGA GTC CAA ACC GGA CT	Em5	GAC TGC GTA CGA ATT AAC
Me12	TGA GTC CAA ACC GGA GG	Em6	GAC TGC GTA CGA ATT GCA
Me13	TGA GTC CAA ACC GGA AA	Em7	GAC TGC GTA CGA ATT CAA
Me14	TGA GTC CAA ACC GGA AC	Em8	GAC TGC GTA CGA ATT CAC
Me15	TGA GTC CAA ACC GGA GA	Em9	GAC TGC GTA CGA ATT CAG
Me17	TGA GTC CAA ACC GGT AG	Em10	GAC TGC GTA CGA ATT CAT
Me18	TGA GTC CAA ACC GGC AT	Em11	GAC TGC GTA CGA ATT GCA
Me19	TGA GTC CAA ACC GGT TG	Em12	GAC TGC GTA CGA ATT CAT
Me20	TGA GTC CAA ACC GCT GT	Em13	GAC TGC GTA CGA ATT CTA
		Em14	GAC TGC GTA CGA ATT CTC
		Em15	GAC TGC GTA CGA ATT CTT
		Em16	GAC TGC GTA CGA ATT GAT
		Em17	GAC TGC GTA CGA ATT ATG
		Em18	GAC TGC GTA CGA ATT AGC
		Em19	GAC TGC GTA CGA ATT ACG
		Em20	GAC TGC GTA CGA ATT TAG

Table 3.3. SRAP primers screened (and selected) for this study (Row= Forward Primers, Column= Reverse Primers, Gray=screened and Black=selected)

	Em1	Em2	Em3	Em4	Em5	Em6	Em7	Em8	Em9	Em10	Em11	Em12	Em13	Em14	Em15	Em16	Em17	Em18	Em19	Em20
Me1	Gray	Gray	Gray	Gray	Gray	Gray	Gray	Gray	Gray	Gray										
Me2	Black	Black	Gray	Gray	Gray	Gray	Gray	Black	Gray	Gray										
Me3	Gray	Gray	Black	Black	Gray	Gray	Gray	Gray	Gray	Gray										
Me4	Gray	Gray																		
Me11											Black	Gray	Gray	Black	Black	Gray	Black	Gray	Gray	Gray
Me12											Gray	Black	Gray	Black	Gray	Gray	Gray	Gray	Gray	Gray
Me13											Gray	Black	Gray	Gray	Gray	Gray	Gray	Gray	Gray	Gray
Me14											Gray	Gray	Gray	Gray	Gray	Gray	Gray	Gray	Gray	Black
Me15											Gray	Gray	Gray	Gray	Black	Gray	Black	Gray	Gray	Gray
Me17											Gray	Gray	Gray	Gray	Gray	Black	Gray	Gray	Gray	Gray
Me18											Gray	Gray	Black	Gray	Gray	Gray	Gray	Gray	Black	Black
Me19											Gray	Gray	Gray	Gray	Black	Gray	Gray	Gray	Gray	Gray
Me20											Gray	Gray	Gray	Gray	Gray	Gray	Gray	Gray	Gray	Gray

SRAP analysis

The SRAP analysis were conducted using the 15 µl PCR reactions mixture: 1xPCR buffer [10mM Tris-HCL (pH 9.0 at 25°C), 50 mM KCL and 0.1% Triton X-100, Promega], 1.5 mM MgCl₂, 0.25 mg BSA, 0.2 mM dNTPs, 0.5 mM of each forward and reverse primers (IDT, Coralville, Iowa, USA), 1 unit of Taq DNA polymerase (Promega, Madison, WI, USA), and approximately 10-15 ng of total DNA. All reactions were carried out by a MJ Research DNA Thermocycler or Eppendorf thermal Cycler with the following conditions: 5 min of initial denaturing at 94°C, five cycles of there steps: 1 min of denaturation at 94°C, 1 min of annealing at 35°C and 1 min of elongation at 72°C, followed by further 35 cycles with annealing temperature being increased to 50°C, with a final extension at 72°C for 5 minutes, following Li and Quiros (2010). PCR amplified products were mixed with loading dye and separated on 2% agarose gel, stained with EtBr and visualized with a UV light source. Negative control reactions were run without DNA for all PCR amplifications to ensure reaction components were uncontaminated. Each primer combinations PCR quality was carefully examined by the gel bands and repeated if needed with selected samples to confirm the reproducibility of the genetic markers. Size of amplification products was estimated using the 100 kb ladder (Promega,

Madison, Wisconsin, USA). Final gel products were viewed using Gel Doc XR (BIO-RAD, Hercules, California, USA) and digitally recorded on Quantity One software (BIO-RAD, v.4.5.1).

Data analyses

SRAP markers were scored either present (1) or absent (0). The data were entered into a binary matrix and assessed for the level of polymorphism and expected heterozygosity (assumption made that populations were in Hardy-Weinberg equilibrium) across individuals within each population and then averaged across all markers. Expected heterozygosity (H_e) was calculated for each population in total for each marker as follows:

$$H_e = 1 - (p^2 + q^2)$$

where p is the frequency of the dominant allele and q is the frequency of the null allele. Genetic relationships within and among populations were estimated using the similarity coefficients of Nei and Li (1979) and Principal Coordinates Analysis (PCO) using Gower general similarity coefficients (Gower 1971), were calculated using MVSP 3.0 (Multi-Variate Statistical Package: Kovach Computing Services 1987-2009). Pairwise similarities were averaged for individuals within and among populations. A score closer to 1 would indicate maximum heterozygosity, and more variability in genetic diversity, where a score of 0 would indicate homozygosity (recessive) and a less variable population.

STRUCTURE analysis

A Bayesian algorithm, as implemented in STRUCTURE version 2.3.4 (Pritchard et al. 2000, Falush et al. 2007), was used to define genetic groups within each species. This algorithm infers genetic discontinuities from individual multilocus genotypes without *a priori* knowledge of geographic location or taxonomy. The default settings of the program were used, including an admixture model. To determine the most likely number of groups (K) in the data, a series of analyses were performed from $K = 1$ to 10 (upper limit determined by the number of populations plus three (Evanno 2005)), using a burn-in period and *Markov Chain Monte Carlo* (MCMC) both set at 100,000 repetitions, with ten iterations per K (Porras-Hurtado et al. 2013). These

results were examined using the ΔK method (Prichard et al 2000) to identify the most likely number of groups in the data using STRUCTURE HARVESTER (Earl and vonHoldt 2012).

Results

Polymorphism and genetic diversity

Of 71 individuals, representing 19 *Erythrina sandwicensis* populations, 241 loci were clearly amplified using 20 combinations of 13 forward and 20 reverse primers, and 187 (77.59 %) of these were identified as polymorphic. By island, Hawai‘i Island had a total of 65 polymorphic loci (26.97%), Moloka‘i with 54 (22.41%), O‘ahu with 53 (21.99%), Kaho‘olawe had 49 (20.33%), Maui populations were found to have 42 (17.43%), Kaua‘i had 41 (17.01%), and Lana‘i populations had the least number of polymorphic loci with 26 (10.79%). As a group, the islands of Maui, Lana‘i, and Moloka‘i were found to have 83 polymorphic loci (34.44%). Within populations, polymorphism is low, but comparing across islands, the percentage of polymorphic loci is high. The percentage of polymorphic loci identified in *E. sandwicensis* populations are summarized in Table 3.4. The estimates of genetic diversity, or the expected heterozygosity (H_e), for *E. sandwicensis* populations are summarized in Table 3.4. The total average expected heterozygosity (H_e) for all islands was 0.220. For individual islands, H_e ranged from 0.041 to 0.082, with an overall mean of 0.067.

Table 3.4. Calculated percentage of polymorphic loci and expected heterozygosity (H_e)

Island	N (# of individuals)	% Polymorphism	H_e (expected heterozygosity)
Hawai‘i	16	26.97	0.082
Kaho‘olawe	8	20.33	0.075
Kaua‘i	8	17.01	0.059
O‘ahu	16	21.99	0.071
Maui	8	17.43	0.061
Lana‘i	4	10.79	0.041
Moloka‘i	11	22.41	0.079
Maui, Lana‘i, and Moloka‘i	23	34.44	0.111
Total	71	77.59	0.220

Similarity matrix

Genetic relationships within and among island populations were estimated using similarity coefficients where a calculation of 0 would indicate the least amount of similarity, and a calculation of 1 would equate to complete genetic identity. Similarity between islands ranged from 0.642 to 0.942, the least similarity occurring between the populations on Kaua‘i and Lana‘i and the highest similarity occurring among individuals on Lana‘i (Table 3.5). These data are summarized in Table 3.5, with green highlight representing the least similar populations and turquoise highlighting the most similar.

Table 3.5. Genetic similarities among islands using the similarity coefficients of Nei and Li (1979), where green highlight = least similar and turquoise highlight = most similar

	Hawai‘i	Kaho‘olawe	Kaua‘i	O‘ahu	Lana‘i	Maui	Moloka‘i
Hawai‘i	0.910						
Kaho‘olawe	0.859	0.908					
Kaua‘i	0.712	0.658	0.931				
O‘ahu	0.785	0.778	0.738	0.926			
Lana‘i	0.741	0.706	0.642	0.711	0.942		
Maui	0.760	0.742	0.642	0.742	0.874	0.926	
Moloka‘i	0.748	0.715	0.648	0.729	0.867	0.888	0.915

Principal coordinate analysis

A principal coordinate analysis (PCO) was conducted with samples of individuals from every population (Fig. 3.1). The first axis accounted for 27.0% of the variation, while the second axis accounted for 18.2%. Data is also displayed with all populations on each island identified (Fig. 3.2). The populations of a single island generally clustered together on the graph and were separated from other island populations with no outliers. The first axis clearly distinguishes individuals from Maui, Moloka‘i and Lana‘i from those of the other islands, these all forming a tight grouping. The second axis then distinguishes the individuals on Kaua‘i from those on O‘ahu and those on Hawai‘i and Kaho‘olawe. Figure 3.2, however, depicts these populations individually as each island separates from one another. O‘ahu populations clustered together in the third quadrant, but far above the populations of Kaua‘i (also in the third quadrant) along the second axis. Interestingly, the populations of Hawai‘i and Kaho‘olawe clustered together in the

fourth quadrant. Figure 3.4 more distinctly depicts these islands' populations separating from each other. An in-depth explanation for this peculiar grouping is offered in the discussion.

STRUCTURE

Four genetic groups were identified as best fitting the data with STRUCTURE analysis. From nineteen geographically distinct locations on seven Hawaiian Islands (from which these samples were collected), four genetic populations emerged (Fig. 3.5). These four groups correspond to the four island clusters identified in the PCO analysis: O'ahu populations form one group, Kaua'i populations form another, Hawai'i and Kaho'olawe populations form one group, and populations from Maui, Moloka'i, and Lana'i comprise the last group. Not only does this correlate precisely with the PCO graphs, but also with geographic data.

Discussion

There is a great amount of genetic variation within *E. sandwicensis* across the Hawaiian Islands. Overall polymorphism is high, and comparable to the highest levels found within a single species (Morden 2011). However, when plants within islands and within populations are compared, the level of polymorphism decreases dramatically. Individual islands had low rates of polymorphism, which suggests more uniform populations within each island. The data do not, however, imply an inbreeding depression is occurring due to the uniformity among all islands. If one island was highly polymorphic, while others were not, this might suggest an inbreeding depression was occurring.

The measures of expected heterozygosity are also low when compared island by island. The total average expected heterozygosity across all islands was low as well (0.22) which indicates these populations are less variable, genetically, and possess recessive alleles. This is concurrent with observations in the field. Populations of wiliwili are usually small, isolated, and fragmented, with (apparently) little gene flow occurring. Each population is, therefore, unique; possessing their own unique set of homozygous alleles.

The similarity matrix and the PCO show the two islands that are least similar, genetically, are Kauai'i and Lana'i, though Maui is also quite distinct from Kaua'i as well. This is depicted

visually on the PCO graph for every population (Figs. 3.1 and 3.2) where Kaua'i is located further from every island, but especially distanced from Maui Nui. In an evolutionary context, these populations must have been genetically distanced from each other, with little or no gene flow occurring throughout their evolution in the islands.

The greatest amount of genetic similarity will generally occur when individuals on an island are compared (Kwon and Morden 2002, Loeffler and Morden 2003). The high similarity among Lana'i plants is consistent with only the one population examined with four individuals sampled. Comparing inter-island similarity values, the island group of Maui, Lana'i, and Moloka'i are most similar to each other. These islands were formally one large land mass, known as Maui Nui (Price and Elliott-Fisk 2004). Maui Nui also includes Kaho'olawe, but plants on this island have an affinity with those from Hawai'i Island rather than with other Maui Nui plants (see below).

The structure analysis correlates with the similarity matrix and the PCO graphs, with very little admixture occurring in the four genetic groups identified. This is unusual for a Hawaiian endemic taxon. For example, the *Meterosideros polymorpha* complex is just that: complex. A study by Harbaugh et al. (2009) demonstrated the large amount of admixture that can appear in Hawaiian taxa and is especially true when analyzing the microsatellite data of varieties in *M. polymorpha*.

From a conservation perspective, genetic distinctiveness is pertinent to keep in mind when outplanting wiliwili propagules. If a conservation goal is to preserve island uniqueness, seeds and seedlings should be planted on the islands from which they were collected. However, if higher genetic diversity is desired, transplanting one island-type to another island may be beneficial. An introduction of a Lana'i (or Maui) plant to Kaua'i could redefine the genetic diversity of Kaua'i wiliwili. However, an introduction of a Hawai'i plant to Kaho'olawe might not make much difference (in terms of adding genetic diversity), based on the similarity matrix results.

The close relationship of Hawai'i and Kaho'olawe is interesting and warrants further investigation. A possible explanation for this could be found in early Hawaiian history where

propagules of wiliwili were transferred from the island of Hawai‘i to Kaho‘olawe for the species’ valuable wood (pers. comm., LeGrande, M., 07 March 2018). In these times, wiliwili wood was valued for its buoyancy, being used for floatation and fishing devices, as well as for surfboards (papa he’e nalu) (Rock 1913; Medeiros et al. 1998; Staples and Herbst 2005). PCO analysis of Hawai‘i and Kaho‘olawe (Fig. 3.4), suggests that this may have occurred from the Waikoloa populations to Kaho‘olawe populations since they are, genetically, most similar. However, the variation in the Kaho‘olawe plants is much greater than within Hawai‘i Island populations. Two decades ago, the Kaho‘olawe Island Reserve Commission (KIRC) was outplanting propagules of wiliwili on Kaho‘olawe that was sourced internally, from elsewhere in Maui Nui, or possibly from elsewhere in the archipelago if the supply of wiliwili propagules was insufficient (Kaho‘olawe Island Reserve Commission 1998).

From these data, it appears as though the dispersal of wiliwili did follow a biogeographical pattern. Trees on Kaua‘i are relatively unarmed, having a smooth trunk compared to those on other islands. The logically proposed closest relative to wiliwili, *E. tahitensis*, shares this characteristic in their trunks as well. Although not tested here, this is consistent with the colonization of the ancestor of wiliwili to Kaua‘i with subsequent colonization to progressively younger islands, consistent with the progression rule (Hennig 1966, Funk and Wagner 1995).

Future research should investigate characteristics that may distinguish these island populations further. If significant morphological, or other, differences are discovered, this may warrant taxonomic changes at the subspecific level. In addition, *E. sandwicensis* should be included in a phylogenetic study of the genus *Erythrina* to determine its relationship to congeners, and to elucidate if *E. tahitensis* is truly the closest relative.

Figure 3.1. PCO based on SRAP marks for individuals of Hawaiian *Erythrina sandwicensis* from all islands. The first axis accounts for 27.0% and the second axis 18.2% of the total variation (45.2%).

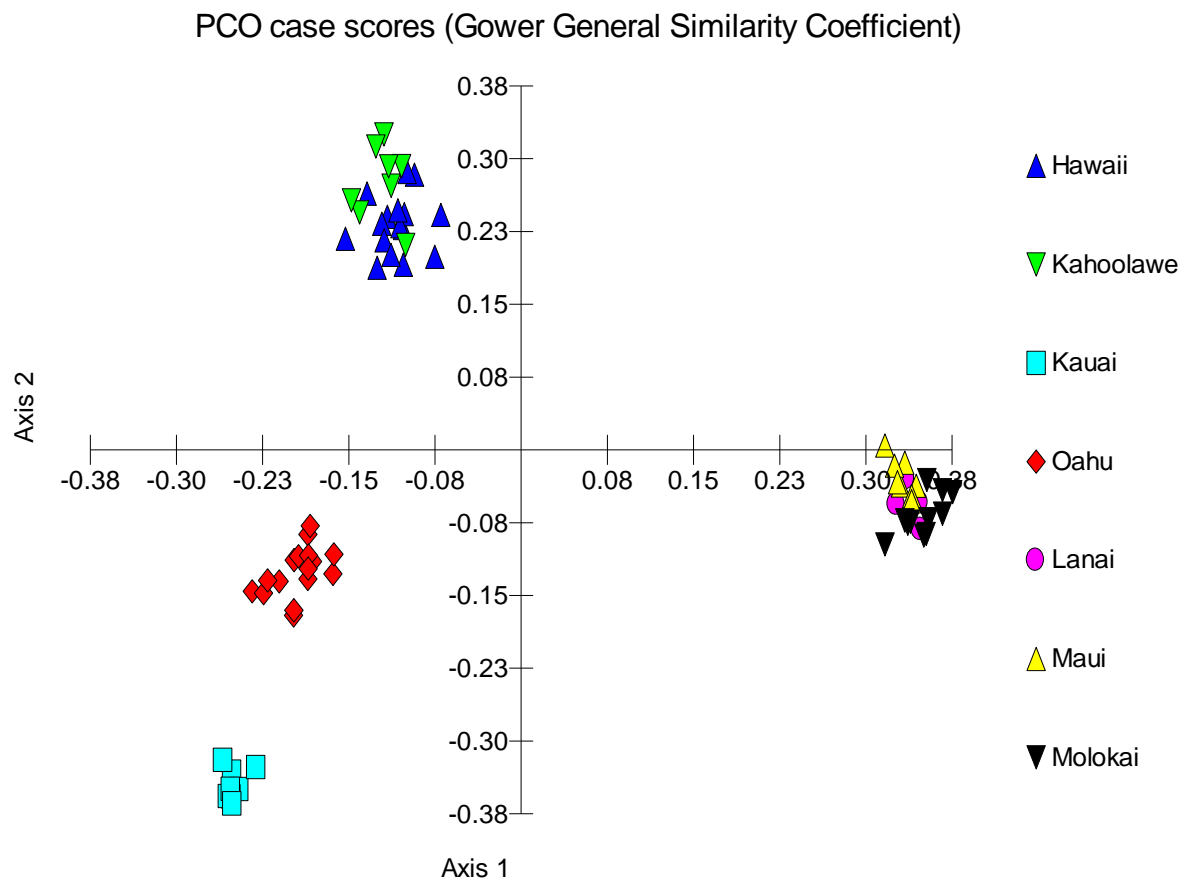


Figure 3.2. PCO based on SRAP marks for individuals of Hawaiian *Erythrina sandwicensis* from all island populations. This is the same PCO as Fig. 3.1, but with each population identified.

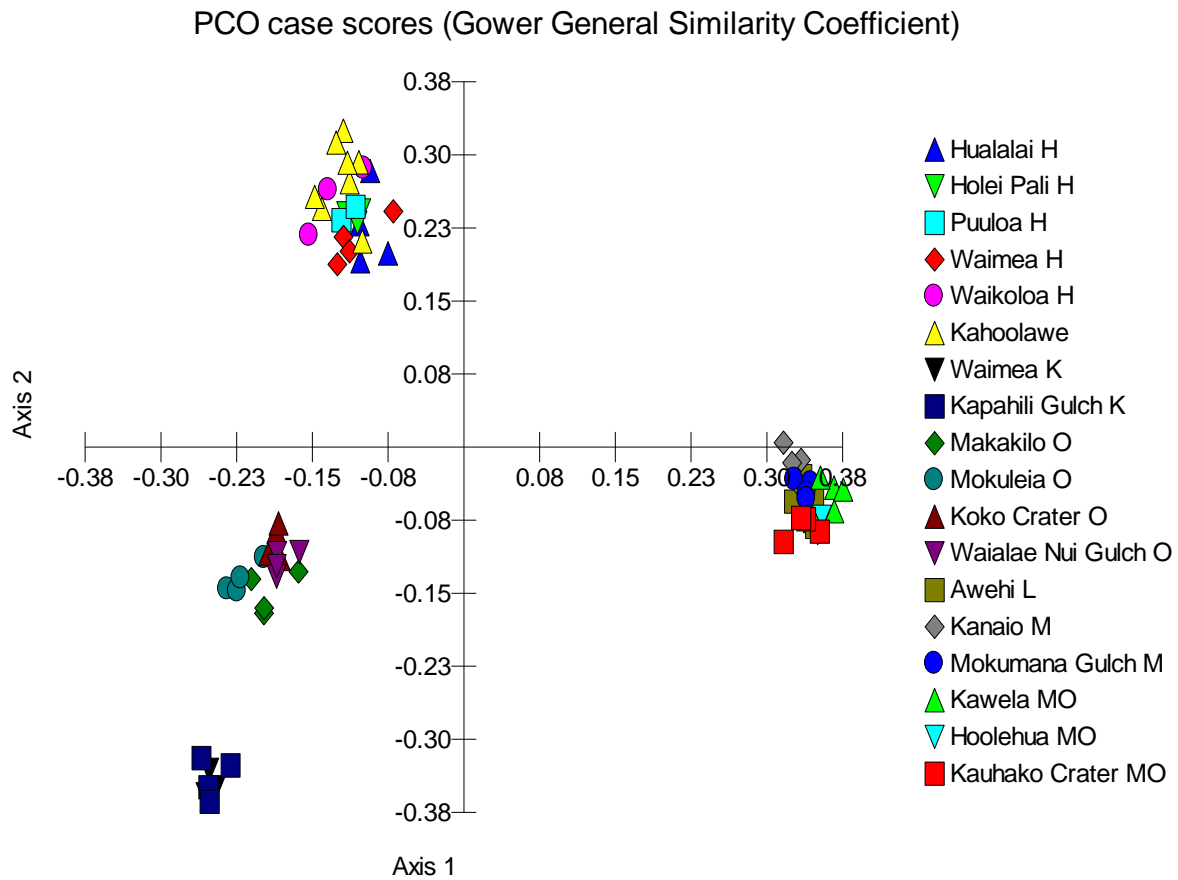


Figure 3.3. PCO based on SRAP marks for individuals of Hawaiian *Erythrina sandwicensis* from Maui, Lana'i, and Moloka'i. The first axis accounts for 19.8% and the second axis 16.9% of the total variation (36.7%).

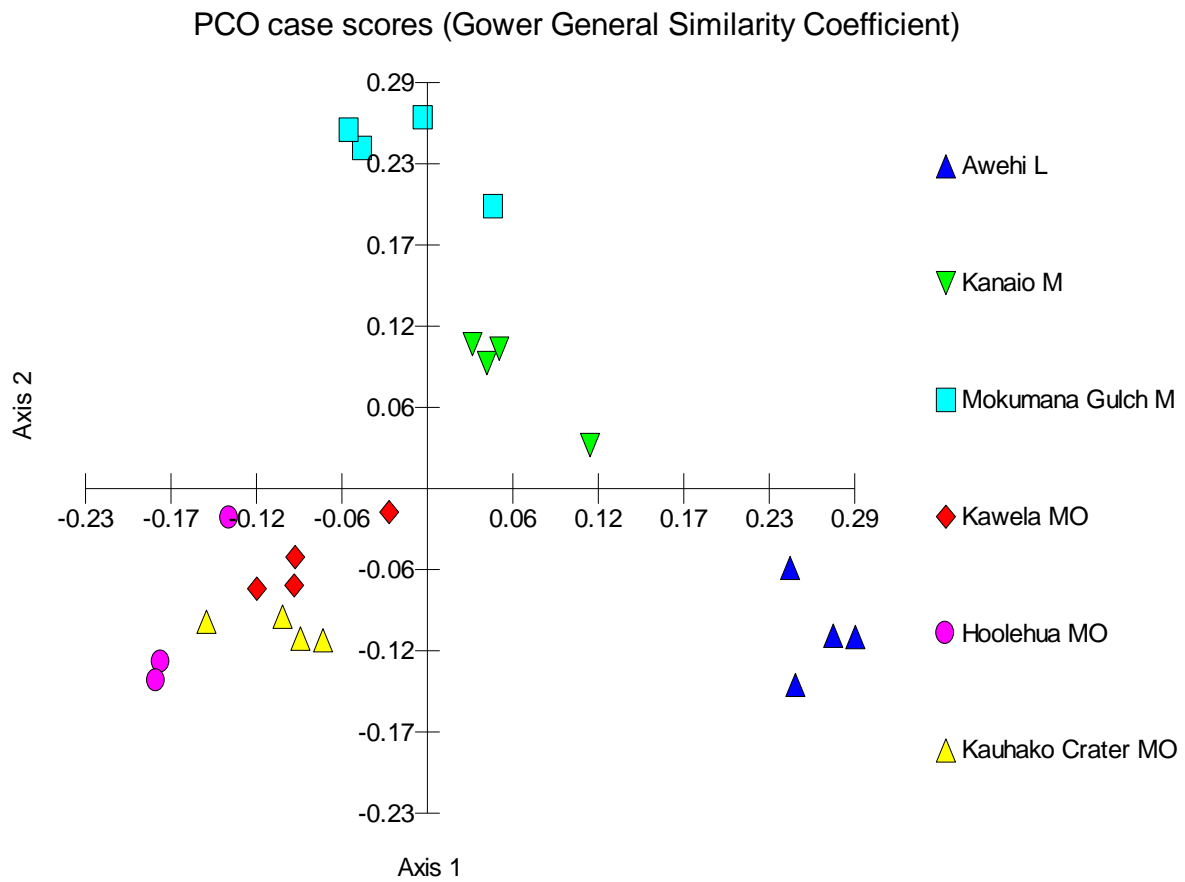


Figure 3.4. PCO based on SRAP marks for individuals of Hawaiian *Erythrina sandwicensis* from Hawai'i and Kaho'olawe. The first axis accounts for 27.5% and the second axis 11.6% of the total variation (39.1%).

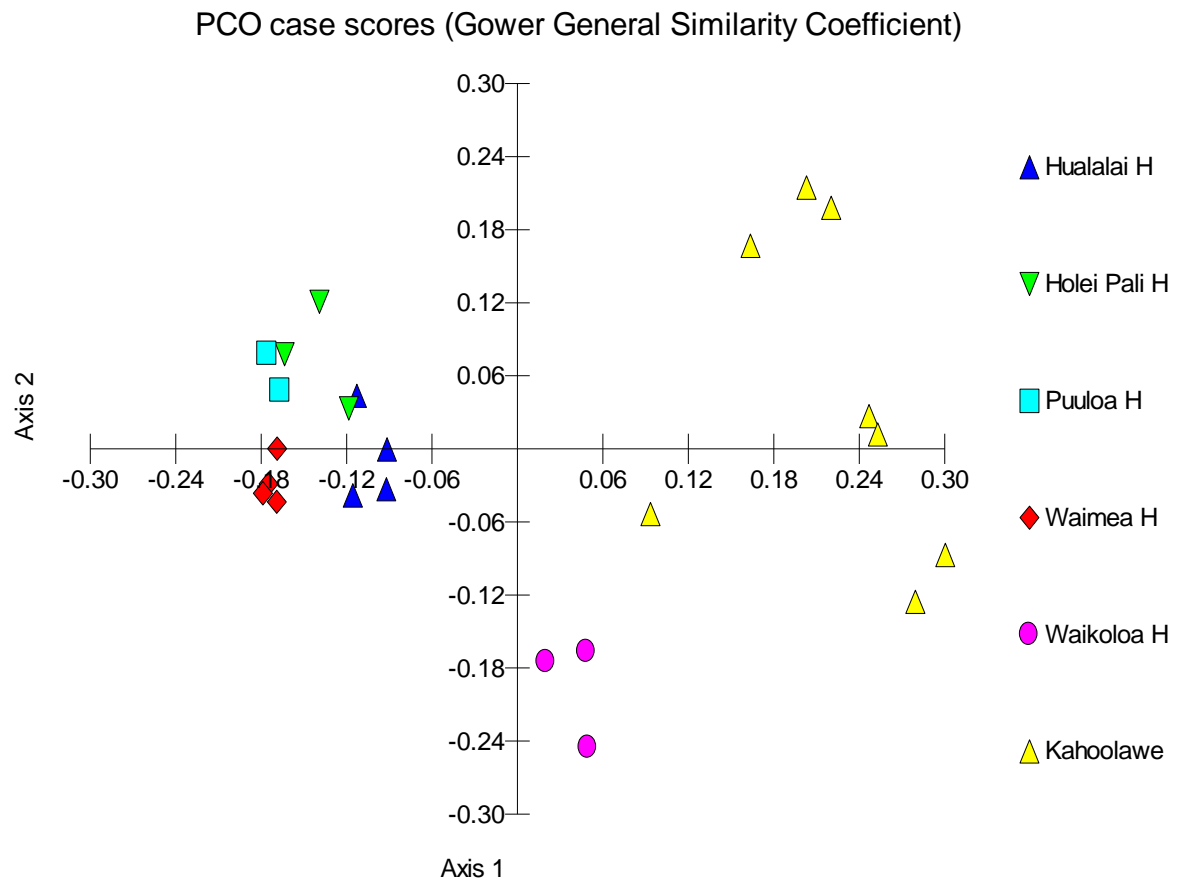
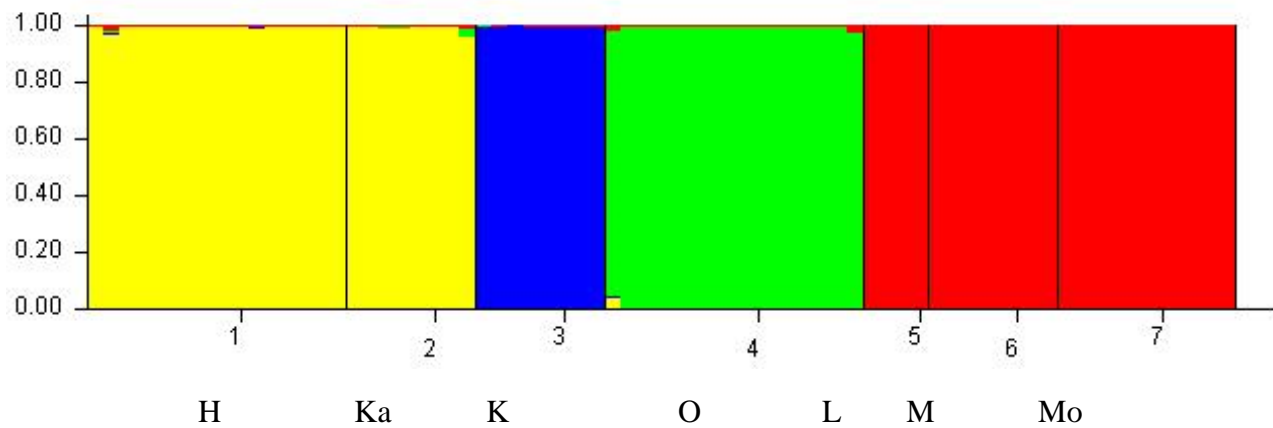


Figure 3.5. Genetic STRUCTURE bar graph of 71 *Erythrina sandwicensis* individuals from all islands based on SRAP data. Individuals and populations are grouped by island and colored segments represent the individual's probability of belonging to a particular group (K).

(1) Hawai'i Island (H); (2) Kaho'olawe (Ka); (3) Kaua'i (K); (4) O'ahu (O); (5) Lana'i (L); (6) Maui (M); (7) Moloka'i (Mo).

$K = 4$. Graphs represent one of 10 iterations from the indicated K value for each species.



CHAPTER 4. DEMOGRAPHIC STRUCTURE AND PATTERNS OF SEEDLING RECRUITMENT IN *ERYTHRINA SANDWICENSIS*

Abstract

Tropical dryland forests are imperiled globally, and the trend is consistent in Hawai‘i. One of the most iconic species in Hawaiian dry forests is the endemic coral tree, *Erythrina sandwicensis*, or wiliwili. Little was known in regard to the regeneration status of this culturally important tree. The aim of this study was to collect demographic data from nine populations of wiliwili on two islands to elucidate wiliwili’s recruitment patterns. In each population, I recorded the slope, the aspect, the elevation, whether the population was fenced or not, and the height or diameter at breast height (DBH) of every individual found. I also measured the level of non-native insect infestation, non-native grass cover, and the percentage of canopy cover plus the percentage of canopy cover that consisted of non-native species. I found little-to-no regeneration occurring in these populations. The only seedlings and saplings recorded were in populations that were fenced, managed for grass, or both. Large numbers of trees should be planted to increase regeneration and avoid these populations becoming more and more isolated.

Introduction

More than half of native Hawaiian plant species (about sixty percent) are federally listed as endangered, and twenty-five percent of these species occur in dry lowland, or scrubland ecosystems (Brueggemann 1996; Sakai et al. 2002). A study by Pau et al. (2009) found that forty-five percent of taxa in these systems are at risk of becoming endangered. One of the most iconic species that occurs in these habitats is the Hawaiian wiliwili, Hawaiian Coral Tree, or *Erythrina sandwicensis* (O. Degener). Wiliwili is the only Hawaiian endemic species in a genus of one hundred fifteen species, and is a member of Fabaceae, or the legume family (Wagner et al. 1999).

Hawaiian wiliwili once dominated the landscape of lowland elevations, up to six hundred meters (Rock 1913; Medeiros et al. 2008). Its distribution is primarily restricted to the hottest, driest environments of the leeward sides on all of the main Hawaiian Islands (Rock 1913; Staples and Herbst 2005). It is a summer-deciduous tree, gaining its leaves in the wet season and

shedding them right before flowering which usually occurs in early spring to July, when the tree is completely (or partially) leafless (Rock 1913; Staples and Herbst 2005). As abundant as this tree was, it is much less common today than in the past (B. P. Koebele 2016, personal communication, 23 December). There is very little recruitment observed in these remaining populations, if any, due to a number of limiting factors discussed below (Kaufman et al. 2014). Currently, wiliwili is one of seven Hawaiian dry forest taxa that is at risk of endangerment, which Pau et al. (2009) attribute to having hermaphroditic, conspicuous flowers, dry fruit that require autochorous dispersal methods, and a large range over more than five islands.

Assessing the demographic structure of a population can give insight into the status of its regeneration (Gurevitch et al. 2006). One such assessment elucidated that less than one percent of native dry forest species are naturally regenerating even in an area where invasive plant species and browsing ungulates were removed (Cordell et al. 2008). However, invertebrates may be important limiting factors, and the introduction of exotic insects is very difficult to manage. Although largely unseen to the human eye, the effects they have on the native flora can be substantial (Ceballos et al. 2002; Messing et al. 2007; Hue et al. 2008; Medeiros et al. 2008; Messing et al. 2009; Gumovsky and Ramadan 2011; Bell et al. 2013; Kaufman et al. 2014). An estimated twenty new arthropods are introduced, and subsequently become established in Hawai'i every year (Messing et al. 2007). For wiliwili, some of these introductions have been devastating. The host-specific Erythrina Gall Wasp, *Quadrastichus erythrinae*, was discovered on the island of O'ahu in April 2005 where it spread to adjacent islands in just four months (Bell et al. 2013). This pest causes substantial defoliation and, in the worst scenarios, tree mortality in just a few months (Kaufman et al. 2014). The biological control agent (*Eurytoma erythrinae*), released as a parasitoid of this wasp in 2008, has been somewhat successful in mitigating these effects (Messing et al. 2009; Bell et al. 2013; Kaufman et al. 2014). However, a 2012 state-wide census revealed that an estimated 30-35% percent of wiliwili trees had died due to the gall wasp attack (Kaufman et al. 2014).

Another introduced arthropod to the Hawaiian Islands that could limit recruitment of wiliwili is the African bruchid beetle, *Specularius impressithorax*. In 2001, the beetle was observed and collected in Makaha Valley on O'ahu island. It took only two years for this seed-

predating beetle to become established on every main Hawaiian island (Medeiros et al. 2008). A study by Medeiros et al. (2008) found that the bruchid beetle accounted for seventy-seven percent of mean seed crop loss in twelve wiliwili populations, on six of the main islands, in the first three years since its introduction. This is because one single larva can reduce the germination rate of the seed in a pod by ninety-seven percent (Medeiros et al. 2008). A biological control endoparasitoid, *Entedon erythrinae*, probably accidentally arrived in the islands with the bruchid beetle. However, with a low rate of recovery for this parasitoid, it may not be an effective control agent in this new, Hawaiian environment (Kaufman et al. 2014). The gall wasp and bruchid beetle together may have catastrophic impacts on reproduction in wiliwili populations (Gumovsky and Ramadan 2011).

There are still other factors that might be influencing the level of regeneration in many wiliwili populations. Invasive grasses inhibit the germination of seeds by covering the landscape and preventing sufficient light levels from reaching the soil (Cabin et al. 2002). These grasses fuel fires that destroy native dryland plants and habitats, and they are able to regenerate after such disturbances, actually thriving in a burnt landscape (Cabin et al. 2002). Grasses and other invasive plant species tend to alter the natural energy fluxes within ecosystems as well as the quality of available resources and habitat (Traveset and Richardson 2006).

A quantitative demographic study of wiliwili populations had yet to be conducted on the islands of O‘ahu and Hawai‘i. Currently, there is no information on regeneration patterns after the gall wasp damage, though one study has looked at the effectiveness of the biocontrol in place (Bell et al. 2013). The goal of this project was to collect demographic data that can be used for conservation management of wiliwili. Therefore, the following research questions and hypotheses were addressed in this experiment:

1. What is the regeneration status of wiliwili populations on O‘ahu and Hawai‘i?
2. What are the limiting factors of recruitment?

Due to the aforementioned pressures on the regeneration of this species, I hypothesized that population structure is skewed toward mature individuals, with very little recruitment.

I hypothesized that populations with high levels of non-native grasses and non-native insect predators (the African bruchid beetle and the *Erythrina* Gall Wasp) would show the lowest recruitment.

Methods

Study sites

My population structure research was conducted at five field sites on the leeward side of O‘ahu, Hawai‘i: Keālia (Mokulē‘ia Forest Reserve), Mākua Kea‘au Forest Reserve, Kalaeloa Heritage Park, Koko Crater Botanical Garden, and Koko Crater’s wild population (City and County of Honolulu Conservation District). I included populations that were well-known as being some of the largest populations of wiliwili on O‘ahu, easily accessible, and with varying degrees of management for grasses and ungulates. I also conducted demographic research on the leeward side of the Big Island (Hawai‘i) at three sites: Waikoloa Dry Forest Preserve, Pālamānu Preserve, and La‘i ‘Opua Dryland Preserve. These sites represent more than half of the known populations on the Big Island. This study took place from May 2017 to August 2017.

Experimental design

To determine the status of wiliwili populations, a demographic study was conducted at all sites mentioned above. The number and size of all trees and seedlings in the population was counted, measured and recorded. The height of trees shorter than 1.5 m, and smaller than 1 cm was recorded, as was the diameter at breast height (DBH) for trees taller than 1.5 m tall, and larger than 1 cm in diameter. Mākua Kea‘au’s population was too large to record every tree; therefore, one 35 m x 35 m plot was randomly placed such inside the predator exclusion fence and one outside the fence. All trees and seedlings that occurred within these plots were measured and recorded. A total of 366 trees (including seedlings and saplings) were counted in this study.

To identify potential factors that may explain observed differences in regeneration, several abiotic and biotic factors were recorded including: slope, aspect, elevation, overall canopy cover, the percentage of non-native canopy cover, the level of understory grass cover, and the levels of gall wasp infestation and bruchid beetle predation. Overall canopy cover,

percentage non-native canopy cover, gall wasp and bruchid beetle infestation were all visually estimated using a scale of 1-25%, 26-50%, 51-75%, >75%. For gall wasp infestation, up to 20 shoots per tree were assessed and scored using this scale regarding percentage of leaf tissue galled. For beetle infestation, this scale was used in reference to the seeds collected on the ground, not in pods. Since the phenology (leaf and flowering cycles) varied at each site, gall wasp data was recorded on structures that were present at the time of the experiment. The gall wasp infests leaves as well as reproductive tissues. Some trees were completely defoliated, but had reproductive structures, and others had only leaves.

Given the small number of sites where wiliwili populations are found, I was unable to carry out statistical analyses to test the effects of biotic and abiotic factors on seedling and sapling regeneration. Therefore, the results are presented descriptively.

Results

Wiliwili was often observed growing on slopes of dry forest habitats. Slopes ranged from almost flat at Kalaeloa (0.3°), to a very steep incline of 51.3° at Keālia. Populations ranged in elevation from almost sea level (8 m) at Kalaeloa, to 320 m at the top of the Keālia Trail with an average of 194 m (± 79 m) elevation (Table 4.1).

Non-native grasses such as Guinea grass (*Urochloa maxima*), or fountain grass (*Pennisetum setaceum*) on Big Island, were in abundance at every site on O‘ahu and Big Island except Koko Crater. Grass cover ranged between 0 (in Koko Crater Botanical Garden) to 100% (outside the fence at Mākua Kea'au) with a mean of $47 \pm 32\%$. Canopy cover, within 5 meters of each tree, ranged from 38% (at Waikoloa Dry Forest Preserve) to 77% (on the slope outside Koko Crater Garden) with an average of $58 \pm 11\%$. The percentage of non-native canopy cover across all sites ranged from 0 (at Waikoloa) to 60% (outside Koko Crater) with a mean of $30 \pm 19\%$.

The Erythrina Gall Wasp was present at every site except the population outside Koko Crater. Some measurements were taken on leaves and some on reproductive structures (pods and flowers). Gall wasp infestation ranged from 0 (outside Koko Crater) to 66.7% (in Waikoloa) with

a mean of $19 \pm 18\%$. Bruchid beetle damage to seeds could only be recorded in Waikoloa due to the phenology of the trees at the time of this study. Trees in the Waikoloa Preserve were the only trees with seeds in pods on the trees, or seeds on the ground. The quantity of afflicted seeds was about 16.7% of all that had fallen to the ground. Seeds still in pods on the trees were not accessible for evaluation. Another herbivore was observed on the wiliwili trees at Pālanuanui; the Chinese rose beetle (*Adoretus sinicus*). Rose beetle damage was recorded on two trees, one of which was considerably defoliated (~35%). These trees were roughly the same size and approximately 20 m apart from each other. Power mildew (*Oidium caricae*) was also observed on one tree at Pālanuanui. Approximately one third of the leaves on this tree were covered with this white, pathogenic fungus. The older leaves were affected the most, with about two thirds of each leaf being coated. These abiotic and biotic factors are presented in Table 4.1.

Table 4.1. Site-specific environmental factors (no scale) and mean of abiotic/biotic factors (Scale: 0-100)

Factor	Kalaeloa	Keālia	Koko Crater (garden)	Koko Crater (wild)	Mākua Kea'au (inside)	Mākua Kea'au (outside)	Pālanuanui	La'i 'Opua	Waikoloa
Slope (°)	0.3	51.3	2.9	48.2	47.7	47.2	Upper=48.6 Lower=11.3	47.9	45.4
Aspect	220°SW	20°NE	320°NW	320°NW	12°N	71°E	275°W	280°W	300°NW
Elevation (m)	8	Upper=320 Lower=56	103	131	238	297	Upper=257 Lower=194	188	213
Fencing	No	No	No	No	Yes	No	Upper=Yes Lower=No	No	Yes
% Non-native grass	32	49	0	52	97	100	Upper=48 Lower=59	16	28
% Canopy cover	52	73	64	77	53	52	Upper=39 Lower=64	64	38
% non-native canopy cover	53	43	29	60	22	13	Upper=9 Lower=16	38	0
% Gall wasp (leaves)	10.4	5	16.8	0*	15.9	17.5	Upper=15 Lower=27	20	25
% Gall wasp (flowers/pods)	NA	NA	NA	NA	NA	NA	NA	NA	66.7
% Bruchid beetle	NA	NA	NA	NA	NA	NA	NA	NA	16.7

*defoliated, very few leaves to measure wasp damage, and no flowers/pods

The structure of populations on O‘ahu and Hawai‘i is heavily skewed toward mature individuals (Fig. 4.1). Saplings, under 150 cm tall, were only found at 4 of the 9 sites (Koko Crater Garden, Pālamanui, La'i 'Opua, and Waikoloa), and saplings, between 1 and 5 cm DBH, were identified in 3 of the 9 sites (Koko Crater Garden and wild, and inside the fence at Mākua Kea'au). Juveniles (between 5 and 20 cm DBH) were measured at every site except in Waikoloa. The remaining 199 trees were all mature trees (more than 20 cm DBH). Dead trees that had not yet fallen were also recorded (as juveniles or adults) and were present at every site. These demographics are summarized in Table 4.2.

Table 4.2. Results of wiliwili demographic study: measured trees found in each category. Seedlings were in two categories: under 50 cm and between 50-150 cm. Saplings were defined as trees with DBH of 1-5 cm, and two classes of adults were: 5 to under 20 cm DBH, and greater than 20 cm DBH.

Class size	Kalaeloa	Keālia	Koko Crater (garden)	Koko Crater (wild)	Mākua Kea'au (inside)	Mākua Kea'au (outside)	Pālamanui	La'i 'Opua	Waikoloa
Small seedling (<50 cm)	0	0	5	0	0	0	2	1	1
Seedling (50-<150 cm)	0	0	4	0	0	0	0	0	3
Sapling (1-<5 cm DBH)	0	0	4	3	1	0	0	0	0
Adult 1 (5-<20 cm DBH)	11	26	7	19	27	36	5	12	0
Adult 2 (>20 cm DBH)	11	32	40	29	17	12	20	8	30
% <5 cm DBH to >5 cm DBH	0	0	21.7	6.3	2.3	0	8	5	13.3
Standing dead (adult)	8	30	2	16	5	6	9	8	9
Standing dead (juvenile)	3	4	1	1	0	0	0	3	4

Discussion

Most of the populations observed had very little recruitment occurring, if any. While four sites had seedlings (albeit very low numbers), only one of these had individuals that were 1-5 cm DBH. This suggests that the seedlings are not making it to the sapling stage. All three of the sites that did have saplings, had very few of them, and two of these sites had no individuals < 1cm DBH. Although regeneration is expected to be periodic in dry forest environments – dependent on the occurrence of one or more wet years – this suggests many years of little, or no, recruitment. Only the Koko Crater Garden site had seedlings and saplings, and even there the numbers were very low compared to the number of adults.

Regeneration was recorded in sites that were fenced, managed for grass, or both. For example, small seedlings were only recorded in the upper portion of the fenced Pālamanui Preserve. It should be noted that inside the fence there was management for grass, but fountain and guinea grass were abundant outside the fence. Koko Crater Botanical Garden is not fenced but is naturally excluded from a large amount of seedling predators being inside a crater and a popular tourist attraction. The garden is managed for invasive grasses and irrigated as well. Waikoloa Dry Forest Preserve is also fenced, partially irrigated, and managed for grass, being the second leading site with natural regeneration. Wild sites (Kalaeloa, outside Koko Crater, Mākua Kea'au, La'i 'Opuā, and Keālia) had the least amount of recruitment occurring, and the highest recorded levels of invasive grasses. In a study by Sylva (2014), it was demonstrated that the removal of invasive grass (in this case, fountain grass on Hawai'i Island) can have a significant effect on the natural recruitment of wiliwili seedlings. Upon returning to Kea'au after winter rains, some recruitment was observed, suggesting that a limiting factor is seedling survival. The amount of native and non-native canopy cover did not seem to affect regeneration in any site.

The Erythrina Gall Wasp (*Quadrastichus erythrinae*) (EGW) was observed at every site, except the wild population outside Koko Crater (wild). This is because, at the time of the study, most of the trees at this site were defoliated and had not yet produced reproductive structures. Therefore, no gall wasp data could be taken. In general, “galling” on vegetative tissues was less

than on reproductive structures like flower buds and floral tissue and seed pods. The most significant damage to these procreant tissues was recorded in Waikoloa. During the timeframe of this study, this was the only site where seed pods were abundant. The range of damage, on the ten trees data was collected from, was between 50-99%. One tree was particularly infested, with nearly every seed pod “galled” so severely that seeds contained inside were dwarfed and shriveled. The probability of these seeds germinating decreases as “galling” severity increases (Kaufman et al. 2014). Galling was present more often on reproductive structures than on vegetative, suggesting the current biocontrol (*Eurytoma erythrinae*) may be more effective on leaves than flowers and pods. A new parasitoid wasp, used as a biocontrol, (*Aprostocetus nitens*) for the EGW is undergoing an environmental assessment at this time and is believed to specialize on gall wasps attacking the reproductive structures (Kaufman et al. 2014). It appeared as though some trees were hit harder than others, and this is consistent with the findings of Bell et al. (2013) who found that trees in close proximity to each other experienced similar effects from “galling” (in Waikoloa). This study also found that drought conditions may also increase the susceptibility of wiliwili trees (Bell et al. 2013). In the context of climate change, with a recent drying trend occurring statewide (Frazier and Giambelluca 2017), the health of these trees may be in jeopardy. A decrease in rainfall might render the trees even more defenseless against the EGW without effective biocontrol agents in place. The high amounts of standing dead in two of the wild sites (Keālia and Koko Crater’s wild population) may have been due to the EGW, as 30-35% of trees fell victim to this pest before 2012 (Kaufman et al. 2014).

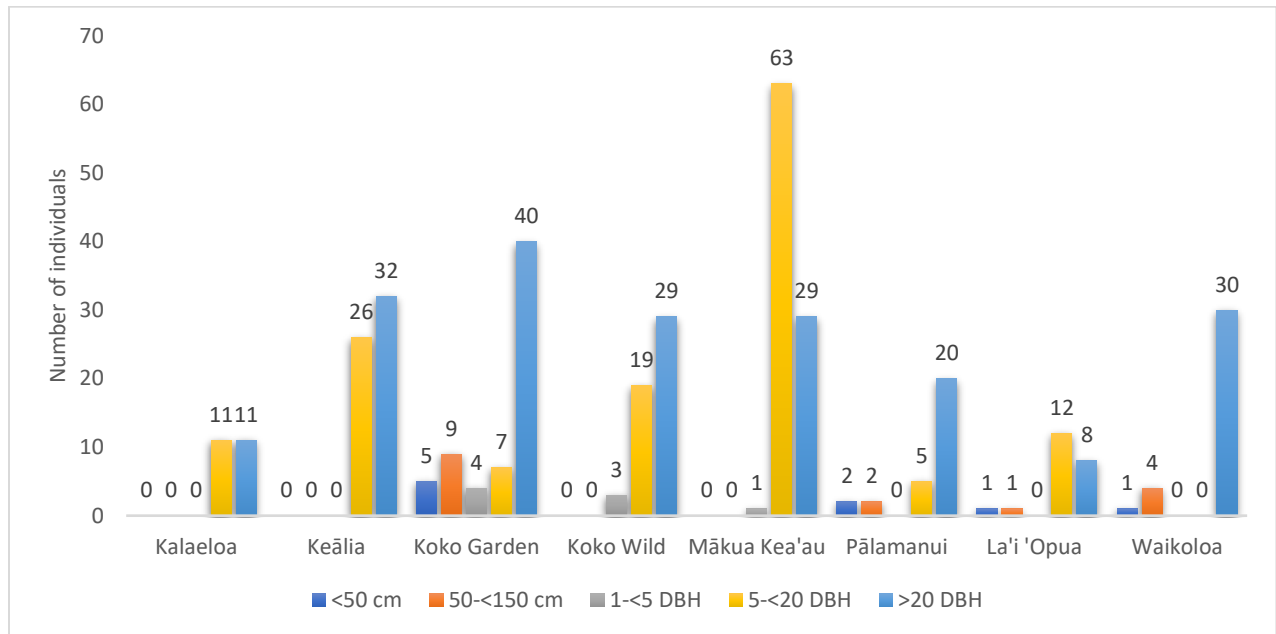
Waikoloa Dry Forest was the only site with an abundance of seeds available for bruchid beetle data collection. However, after the study’s conclusion, I revisited some of these populations and noticed bruchid damage on the seeds. In Waikoloa, an average of five out of every thirty seeds on the ground were infested with bruchid larvae, some with exit holes. In Koko Crater Garden, I noticed roughly the same amount of infestation. It appears as though there is a window of time where seeds in pods are not yet damaged, in the first few weeks of the seed pod cracking open. Pods that had been open for several weeks were heavily infested, where newly opened pods had very little, if any, damage. This is consistent with the conclusions of Kaufman et al. (2014) where they found only 18% of newly opened pods were infested, but this

rate increased dramatically with time. At the end of a fifteen-week period, over 80% of pods contained bruchid beetle larvae. This directly affects the natural regeneration of this species as seeds found with just one exit hole reduced germination rates to 10%, and seeds with more than one exit hole did not germinate. The endoparasitoid biocontrol for the beetle, *Entedon erythrinae*, was only found in 8% of seedpods collected, suggesting that it may not be an effective measure against this ubiquitous seed predator (Kaufman et al. 2014). Germination rates of seeds, without any bruchid beetle damage, from this study were roughly 73% (Chapter 2).

The results of this study indicate that invasive species pose a serious threat to the health of these trees. Mature seeds should be collected as soon as possible (when the pods first open) for restoration efforts. Managing for invasive grasses, insects, and ungulates could benefit the remaining populations of wiliwili well into the future. Because these recorded populations are generally small and isolated, larger numbers of trees should be established to prevent these populations from becoming further fragmented.

Results of this study highlight the lack of recruitment occurring in some of the last remaining, wild populations of wiliwili on O‘ahu and Hawai‘i islands. Only sixteen seedlings (under 150 cm tall) were observed in nine populations on two islands, and only eight saplings (1- <5 cm DBH) were found in just two of the wild populations. This suggests there are bottlenecks in the seed to seedling transition, but also in the seedling to sapling transition. The seed to seedling transition could be due to lower seed output, potentially due to the effects incurred from the gall wasp and bruchid beetle, but also because of the lack of pollinators that allow for cross pollination (Chapter 2), or the loss of dispersers to scarify the seeds. The second bottleneck, in the seedling to sapling transition, may be due to grass cover and ungulates, or it may be related to drought. In Chapter 2, I show that seed mass is a driver of germination probability, growth rate, and seedling height. Although it was beyond the scope of this study, seed mass may be correlated with environmental conditions, such as water availability (Chapter 2). Therefore, we can expect that with drier conditions, less pollinators, and more isolated individuals we are going to be seeing a low number of seedlings, even without the gall wasp and bruchid beetle.

Figure 4.1. Graph of wiliwili demographic study (with Mākua Kea'au plots, in and outside of fence, combined). Height and DBH are in centimeters (cm).



CHAPTER 5. CONSERVATION OF *E. SANDWICENSIS*

The results of my thesis suggest that wiliwili populations are not regenerating and highlight management practices that can enhance their conservation. Specifically, my results indicate that larger populations of wiliwili should be established through outplanting. Currently, based on my population structure and genetic research, populations are isolated and fragmented (Chapter 3 and Chapter 4). If there are more individuals, there is a greater chance of cross-pollination, and this results in greater seed set and more vigorous seedlings. Non-native species are potentially acting as pollinators of wiliwili flowers. However, seed production in wiliwili is currently pollen limited and could, therefore, benefit from human intervention until larger populations can be firmly established. This species obviously benefits from donor pollen from another tree, cross-pollination, similar to other species in the genus *Erythrina*. Management efforts to promote this cross-pollination will be beneficial to the species, especially ensuring there are more individuals in a population to prevent further isolation. Hand-pollination of flowers on trees that reach such heights may not be possible, cost-effective, or the best use of time. However, outplanting to ensure more generations of wiliwili individuals may be the most time-efficient and cost-effective management strategy.

The garden site yielded a greater number of seeds, potentially due to the availability of water, though this is not possible to verify. With a drying climate, this species may be at the edge of its range and ability to mitigate unseasonal drought conditions. Future research could look at seed germination and survival in wetter climatic conditions, possibly at higher elevations.

Because it has been documented that bruchid beetle infestation rates increase with the amount of time the pod has been open, I recommend that seeds be collected as early as possible. From the beginning of the flowering period, resource managers could collect seeds within the following two months, knowing that pods ripen approximately six to eight weeks from the time of fertilization (Chapter 2). As soon as the pods begin to dehisce, that is the best window for collection.

From a relatively low sample size (71 sampled individuals occurring in nineteen wiliwili populations on seven islands), unique genetic identities were discovered. For outplanting efforts,

genetic material (seeds or cuttings) collected from individual island populations should remain on their respective islands if resource managers wish to preserve the genetic identity of these populations (Chapter 3). If, however, they wish to increase genetic diversity within populations, stock from one island being planted on another could decrease the uniqueness of that population but may avoid the potential for inbreeding. Given that the number of samples per population was relatively low, for future studies, I recommend a higher sample sizes per population, and more populations be sampled.

Wiliwili is not currently on the Endangered Species Act list, but my results highlight that recruitment is not observed frequently (Chapter 4). Conservation efforts should be put into effect now to assure the remaining fragments do not become more and more isolated, eliminating any chance to recover.

APPENDIX

Table A1. Best fit model of the effects of pollination treatment and site on the number of fruit produced per flower (all pollination treatments are in comparison to control 2 [cut inflorescence]; site insignificant; $R^2 = 0.2974$)

Coefficient	Value	Stan. error	Deg. Free.	T-value	P-value
Intercept	-0.8428142	0.5642162	80	-1.493789	0.1392
Auto	-0.5913410	0.6226798	80	-0.949671	0.3451
Geit	0.1004850	0.5167850	80	0.1944426	0.8463
Cross	1.1997926	0.4278047	80	2.8045337	0.0063
Flowers	-0.0039531	0.0646493	80	-0.0611471	0.9514

Table A2. Best fit model of the effects of pollination treatment and site on the number of seeds produced per flower (all pollination treatments are in comparison to control 2 [cut inflorescence]; site insignificant)

Coefficient	Estimate	Stan. error	Z-value	P-value
Intercept	-0.0332	0.9106	-0.04	0.97092
Auto	-0.4658	0.6825	-0.68	0.49490
Geit	0.0842	0.5581	0.15	0.88009
Cross	1.5475	0.4334	3.57	0.00036
Flowers	-0.1066	0.1193	-0.89	0.37157

Table A3. Best fit model of the effects of pollination treatment and site on the number of seeds produced per fruit (all pollination treatments are in comparison to control 2 [cut inflorescence]; site and pollination treatment insignificant)

Coefficient	Estimate	Stan. error	Z-value	P-value
Intercept	-1.797	0.393	-4.57	8.1e-13
Fruit	1.054	0.105	10.02	< 2e-16

Table A4. Best fit model of the effects of pollination treatment and site on the mass of the seed (all pollination treatments are in comparison to control 2 [cut inflorescence]; pollination treatment insignificant; $R^2 = 0.3490$)

Coefficient	Value	Stan. error	Deg. Free	T-value	P-value
Intercept	0.6228456	0.07464991	79	8.343555	0.0000
Makua	-0.1192201	0.04257195	79	-2.800438	0.0064

Table A5. Best fit model of the effect of site and pollination treatment on the probability of germination (all pollination treatments are in comparison to control 2 [cut inflorescence]; pollination treatment insignificant)

Coefficient	Estimate	Stan. error	Z-value	P-value
Intercept	1.599	0.763	2.10	0.0361
Makua	-1.947	0.715	-2.72	0.0065

Table A6. Best fit model of the effect of site and mass on the probability of germination (all pollination treatments are in comparison to control 2 [cut inflorescence]; pollination treatment insignificant)

Coefficient	Estimate	Stan. error	Z-value	P-value
Intercept	-3.459	1.230	-2.81	0.0049
Makua	-1.791	0.715	-2.51	0.0122
Mass	8.863	2.067	4.29	1.8e-05

Table A7. Best fit model of the effect of pollination treatment and site on the time to germinate (all pollination treatments are in comparison to control 2 [cut inflorescence]; site and pollination treatment insignificant; $R^2 = 0.04789$)

Coefficient	Value	Stan. error	Deg. Free.	T-value	P-value
Intercept	1.876405	0.09877497	80	18.99676	0.0000

Table A8. Best fit model of the effect of pollination treatment and site on seedling growth rate (all pollination treatments are in comparison to control 2 [cut inflorescence]; site insignificant; $R^2 = 0.37060$)

Coefficient	Value	Stan. error	Deg. Free.	T-value	P-value
Intercept	-20.993944	8.828902	44	-2.377866	0.0218
Auto	-2.503817	1.974081	44	-1.268346	0.2113
Geit	-0.371677	1.685254	44	-0.220547	0.8265
Cross	0.882690	1.231663	44	0.716665	0.4774
Days alive	0.795498	0.214530	44	3.708090	0.0006

Table A9. Best fit model of the effect of mass, pollination treatment, and site on seedling growth rate ("Control" is the control 2 [cut inflorescence]; pollination treatment insignificant; $R^2 = 0.50320$)

Coefficient	Value	Stan. error	Deg. Free.	T-value	P-value
Intercept	-29.439527	7.517007	46	-3.916389	0.0003
Days alive	0.807524	0.178621	46	4.520874	0.0000
Mass	10.912505	2.668200	46	4.089837	0.0002
Makua	2.058266	0.999760	46	2.058759	0.0452

Table A10. Best fit model of the effect of pollination treatment on final seedling height (all pollination treatments are in comparison to control 2 [cut inflorescence]; site insignificant; $R^2 = 0.1976$)

Coefficient	Value	Stan. error	Deg. Free.	T-value	P-value
Intercept	11.246197	1.395398	45	8.059488	0.0000
Auto	-2.672261	2.175704	45	-1.228228	0.2257
Geit	0.729437	1.824942	45	0.399704	0.6913
Cross	2.692181	1.260997	45	2.134962	0.0382

Table A11. Best fit model of the effect of pollination treatment and site on total leaf area (all pollination treatments are in comparison to control 2 [cut inflorescence]; $R^2 = 0.1400$)

Coefficient	Value	Stan. error	Deg. Free.	T-value	P-value
Intercept	75.73315	15.87306	41	4.771174	0.0000
Makua	2.63667	15.49546	41	0.170157	0.8657
Auto	-30.83275	25.76830	41	-1.196538	0.2384
Geit	28.75268	25.23560	41	1.139370	0.2612
Cross	19.02810	14.56025	41	1.306852	0.1985

Table A12. Best fit model of the effect of pollination treatment and site on seedling basal diameter (all pollination treatments are in comparison to control 2 [cut inflorescence]; site insignificant; $R^2 = 0.2276$)

Coefficient	Value	Stan. error	Deg. Free.	T-value	P-value
Intercept	5.326250	0.2333934	44	22.820914	0.0000
Auto	-0.054917	0.4469143	44	-0.122880	0.9028
Geit	0.418000	0.4042492	44	1.034016	0.3068
Cross	0.846053	0.2601498	44	3.252177	0.0022

Table A13. Best fit model of the effect of mass on seedling height (all pollination treatments are in comparison to control 2 [cut inflorescence]; site and pollination treatment insignificant; $R^2 = 0.22830$)

Coefficient	Value	Stan. error	Deg. Free.	T-value	P-value
Intercept	4.602542	2.408666	48	1.910826	0.0620
Mass	11.506914	3.120815	48	3.687151	0.0006

LITERATURE CITED

- Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, 19: 716–723.
- Aslan, C.E., Zavaleta, E.S., Tershy, B., Croll, D., Robichaux, R.H. Imperfect replacement of native species by non-native species as pollinators of endemic Hawaiian plants. *Conservation Biology*, 28: 478-488.
- Baker, H.G. and Baker, I. 1983. Floral nectar sugar constituents in relation to pollinator type. In: *Handbook of experimental pollination biology*. Scientific and Academic Editions, New York.
- Baraloto, C., Forget, P. and Goldberg, D.E. 2005. Seed mass, seedling size and neotropical tree seedling establishment. *Journal of Ecology*, 93: 1156-1166.
- Bartoń, K. 2013. MuMIn: multi-model inference, R package version 1.9.13. Web. <https://cran.r-project.org/web/packages/MuMIn/MuMIn.pdf>. Retrieved 11 May, 2018.
- Bell, R.C., Belmaker, A., Couch, C.S., Marchetto, K.M., Simonis, J.L., Thomas, R.Q., Sparks, J.P., Brown, J.M., Francisco, K.S. and Manuel, M.E. 2013. Effectiveness of *Erythrina* gall wasp biocontrol and implications for the recovery of threatened Wiliwili trees (Fabaceae: *Erythrina sandwicensis*). *The Journal of the Torrey Botanical Society*, 140: 215-224.
- Bruegmann, M. M. 1996. Hawaii's dry forests. *Endangered Species Bulletin*, 11: 26–27.
- Bruneau, A. 1996. Phylogenetic and biogeographical patterns in *Erythrina* (Leguminosae: Phaseoleae) as inferred from morphological and chloroplast DNA characters. *Systematic Botany*, 21: 587-605.
- Bruneau, A. 1997. Evolution and Homology of Bird Pollination Syndromes in *Erythrina* (Leguminosae). *American Journal of Botany*, 84: 54–71.

- Budak, H., Shearman, R.C., Parmaksiz, I., Gaussoin, R.E., Riordan, T.P., and Dweikat, I. 2004. Molecular characterization of Buffalo grass germplasm using sequence-related amplified polymorphism markers. *Theor. Appl. Genet.* 108: 328-334.
- Burd, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *The Botanical Review*, 60: 83–139.
- Cabin, R.J., Weller, S.G., Lorence, D.H., Flynn, T.W., Sakai A.K., Sandquist, D., Hadway, L.J. 2000. Effects of long-term ungulate exclusion and recent alien species control on the preservation and restoration of a Hawaiian tropical dry forest. *Conservation Biology*. 14: 439–453.
- Cabin, R.J., Weller, S.G., Lorence, D.H., Cordell, S., Hadway, L.J., Montgomery, R., Goo, D., Urakami, A. 2002. Effects of Light, Alien Grass, and Native Species Additions on Hawaiian Dry Forest Restoration. *Ecological Applications*, 12: 1595–1610.
- Campbell, N., Reece, J.B., Urry, L.A., Cain, M.L., Wasserman, S.A., Minorsky, P.V., Jackson, R.B. 2011. *Campbell biology* (9th ed.). Boston: Pearson.
- Ceballos, L., Andary, C., Delescluse, M., Gibernau, M., McKey, D., Hossaert-McKey, M. 2002. Effects of sublethal attack by a sucking insect, *Hyalymenus tarsatus*, on *Sesbania drummondii* seeds: Impact on some seed traits. *Ecoscience*, 9: 28–36.
- Cordell, S., McClellan, M., Carter, Y. Y. and Hadway, L. J. 2008. Towards restoration of Hawaiian tropical dry forests: The Kaupulehu outplanting programme. *Pacific Conservation Biology*, 14: 279–284.
- Cox, P.A. and Elmqvist, T., 2000. Pollinator extinction in the Pacific Islands. *Conservation Biology*, 14: 1237-1239.

- Doyle, J.J., and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.*, 19: 11-15.
- Earl, D.A., and VonHoldt, B.M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4: 359-361.
- Echeveria, A. V. and Alemán, C. E. T. 2005. Reproductive Biology of *Erythrina falcata* (Fabaceae: Papilionoideae). *Biotropica*, 37: 54–63.
- Etcheverry, Ángela V., Figueroa-Castro, D., Figueroa-Flemming, T., Aleman, Maria M., Juarez, Victor D., Lopez-Spahr, D., Yanez, Carolina N., and Gomez, C. A. 2012. Generalised pollination system of *Erythrina dominguezii* (Fabaceae: Papilionoideae) involving hummingbirds, passerines and bees. *Australian Journal of Botany*, 60: 484–494.
- Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14: 2611-2620.
- Faegri, K. and Van der Pijl, L. 1979. The principles of pollination ecology, 3rd ed. Elmsford: Pergamon Press Ltd.
- Falush, D., Stephens, M., Pritchard, J.K. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, 7: 574–578.
- Fournier, D.A., Skaug H.J., Ancheta J., Ianelli J., Magnusson A., Maunder M., Nielsen A. and Sibert J. 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimal Methods Software*, 27: 233-249.

- Funk, V. A., Sakai, A.K., and Richardson, K. 2002. Biodiversity: The interface between systematics and conservation. *Systematic Biology*, 51: 235-237.
- Funk, V. A. and Wagner, W. L. 1995. Biogeographic patterns in the Hawaiian Islands. Pages 379–419 in W. L. Wagner and V. A. Funk, eds. *Hawaiian biogeography: Evolution on a hot spot archipelago*. Smithsonian Institution Press, Washington, D.C.
- Galetto, L., Bernardello, G., Isele, I. C., Vesprini, J., Speroni, G., Berduc, A. 2014. Reproductive Biology of *Erythrina crista-galli* (Fabaceae). *Annals of the Missouri Botanical Garden*, 87: 127–145.
- Gardener, M.C. & Daehler, C.C. 2006. Documenting floral visitors to rare Hawaiian plants using automated video recordings. *Pacific Conservation Biology*, 12: 189–194.
- Gower, J. C. 1971. A general coefficient of similarity and some of its properties. *Biometrics*, 27:857-874.
- Guide of US: America's travel network. 2018. Hawaii weather and climate patterns. Web https://www.hawaii-guide.com/content/posts/hawaii_weather_and_climate_patterns Retrieved 24 June, 2018.
- Gumovsky, A.V. and Ramadan, M.M. 2011. Biology, immature and adult morphology, and molecular characterization of a new species of the genus *Entedon* (Hymenoptera: Eulophidae) associated with the invasive pest *Specularius impressithorax* (Coleoptera: Chrysomelidae, Bruchinae) on *Erythrina* plants. *Bulletin of entomological research*, 101: 715-739.
- Gurevitch, J., Scheiner, S.M. and Fox, G.A. 2006. *The ecology of plants*. Sunderland: Sinauer Associates.

- Harbaugh, D.T., Wagner, W.L., Percy, D.M., James, H.F. and Fleischer, R.C., 2009. Genetic structure of the polymorphic *Metrosideros* (Myrtaceae) complex in the Hawaiian Islands using nuclear microsatellite data. PLoS ONE, 4: e4698.
- Hennig, W. 1966. Phylogenetic systematics. Urbana: University of Illinois Press.
- Heu, R.A. et al. 2008. Erythrina Gall Wasp. College of Tropical Agriculture and Human Resources (CTAHR), University of Hawaii at Manoa, 5: 4–5.
- Huyghe, C., Julier, B., Bolanos-Aguilar, E.D., Ecalle, C., Hacquet, J. 2002. Effect of cultivar and environment on seed yield in alfalfa. Crop Science, 42: 45-50.
- Janzen, D.H. 1988. Tropical dry forests: the most endangered major tropical ecosystem. In: Wilson, E.O. (ed) Biodiversity. National Academy Press, Washington DC.
- Johnson, S.D. and Nicolson, S.W., 2008. Evolutionary associations between nectar properties and specificity in bird pollination systems. Biology letters, 4: 49-52.
- Jordano, P. 1993. Pollination biology of *Prunus mahaleb* L.: deferred consequences of gender variation for fecundity and seed size. Biological Journal of the Linnean Society, 50: 65-84.
- Kaho`olawe Island Reserve Commission. 1998. Kaho`olawe Environmental Restoration Plan. Social Science Research Institute: University of Hawai`i at Mānoa. Web http://kahoolawe.hawaii.gov/plans/restoration_plan.html Retrieved 30 June, 2018.
- Kaufman, L., Yalemar, J., and King, C. 2014. Monitoring the health of the endemic *Erythrina sandwichensis* in Hawaii. *University of Hawaii at Manoa College of Tropical Agriculture & Human Resources, Hawaii Department of Agriculture, and Hawaii Department of Land and Natural Resources*: 1-5

- Kearns, C.A. and Inouye, D.W. 1993. Techniques for pollination biologists. University Press of Colorado.
- Kwon, J. A., and C. W. Morden. 2002. Population genetic structure of two rare tree species (*Colubrina oppositifolia* and *Alphitonia ponderosa*, Rhamnaceae) from Hawaiian dry and mesic forests using RAPD markers. *Molecular Ecology*, 11: 991–1001.
- Li, G., and Quiros F. 2001. Sequence related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging *Brassica*. *Theor Appl. Gent.* 103: 455-461.
- Liao, B., Wang, F., Chen, L., Li, P., Ouyang, K., Pian, R., Liu, M., Que, Q., Zhou, X., Xi, W. and Chen, X. 2016. Population Structure and Genetic Relationships of *Melia* Taxa in China Assayed with Sequence-Related Amplified Polymorphism (SRAP) Markers. *Forests*, 7: 81.
- Loeffler, W. F., and C. W. Morden. 2003. Genetic diversity and biogeography of the Hawaiian cordage plant, Olona (*Touchardia latifolia*; Urticaceae), based on RAPD markers. *Biochemical Systematic Ecology*, 31: 1323–1335.
- McCormack, G. 2007. Cook Islands Biodiversity Database, Version 2007.2. Cook Islands Natural Heritage Trust, Rarotonga. Online at <http://cookislands.bishopmuseum.org>. Retrieved 3/12/17.
- Medeiros, A.C., Davenport, C.F., Chimera, C. G. 1998. Auwahi: Ethnobotany of a Hawaiian Dryland Forest. Cooperative National Park Resources Studies Unit, University of Hawai'i at Manoa: 1–47.

- Medeiros, A.C., Vonallmen, E., Fukada, M., Samuelson, A. and Lau, T. 2008. Impact of the newly arrived seed-predating beetle *Specularius impressithorax* (Coleoptera: Chrysomelidae: Bruchinae) in Hawai'i. *Pacific Conservation Biology*, 14: 7-12.
- Medrano, M., Guitian, P. and Guitian, J., 2000. Patterns of fruit and seed set within inflorescences of *Pancratium maritimum* (Amaryllidaceae): nonuniform pollination, resource limitation, or architectural effects? *American Journal of Botany*, 87(4): 493-501.
- Mehrhoff, L. A. 1996. Reintroducing endangered Hawaiian plants. In: *Restoring diversity. Strategies for reintroduction of endangered plants*. Chapman & Hall, New York: 301-332.
- Messing, R. H., Tremblay, M. N., Mondor, E. B., Footitt, R. G. and Pike, K. S. 2007. Invasive aphids attack native Hawaiian plants. *Biological Invasions*, 9: 601–607
- Messing, R. H., Noser, S. and Hunkeler, J. 2009. Using host plant relationships to help determine origins of the invasive Erythrina gall wasp, *Quadrastichus erythrinae* Kim (Hymenoptera: Eulophidae). *Biological Invasions*, 11: 2233–2241.
- Miles, L., Newton, A. C., DeFries, R. S., Ravilious, C., May, I., Blyth, S., Kapos, V. and Gordon, J. E. 2006. A global overview of the conservation status of tropical dry forests. *Journal of Biogeography*, 33: 491–505.
- Morden, C. W. 2011. Genetic analysis of within and among population variation of *Chamaesyce celastroides* var. *kaenana* . Final Report. Oahu Army Natural Resources Program, Schofield Barracks, O'ahu, Hawai'i.
- Morden, C. W., Caraway, V., and Motley, T.J. 1996. Development of a DNA library of native Hawaiian plants. *Pacific Science*, 50: 324-335.
- Morden, C.W. and Harbin, S.C. 2013. Evolution and Biogeographic Origins of the Endemic Hawaiian Genus *Hesperomannia* (Asteraceae). *Pacific science*, 67: 219-235.

- Nei, M., and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction Endonucleases. *Proc. Natl. Acad. Sci.*, 76: 5269-5273.
- Neill, D. A. 1988. Experimental Studies on Species Relationships in *Erythrina* (Leguminosae: Papilionoideae). *Annals of the Missouri Botanical Garden*, 75: 886–969.
- Neuschulz, E.L. et al., 2016. Pollination and seed dispersal are the most threatened processes of plant regeneration. *Scientific Reports*, 6: 1–6.
- Nicolson, S. W. 2002. Pollination by passerine birds: why are the nectars so dilute? *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 131: 645-652.
- Pau, S., Gillespie, T.W., and Price, J. 2009. Natural history, biogeography, and endangerment of Hawaiian dry forest trees. *Biodiversity and Conservation*, 18: 3167-3182.
- Pender, R.J. 2013. 'Floral trait evolution and pollination ecology in the Hawaiian lobeliad genus, *Clermontia* (Campanulaceae)'. Ph.D. University of Hawaii at Manoa, Honolulu, Hawai'i.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team. 2018. *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-137, <https://CRAN.R-project.org/package=nlme>. Retrieved 11 May, 2018.
- Porras-Hurtado, L., Ruiz, Y., Phillips, C., Carracedo, A., and Lareu, M. 2013. An overview of STRUCTURE: application, parameter settings, and supporting software. *Frontiers in Genetics*, 4: 1–13.
- Prasad, P.V., Boote, K.J. and Allen Jr, L.H., 2006. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [*Sorghum bicolor* (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures. *Agricultural and forest meteorology*, 139: 237-251.
- Price, J. P., and D. Elliott-Fisk. 2004. Topographic history of the Maui Nui complex, Hawai'i, and its implications for biogeography. *Pacific Science*, 58: 27–45.

- Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155: 945–959.
- Raven, P. H. 1974. *Erythrina* (Fabaceae): Achievements and opportunities. *Lloydia* 37: 321-331.
- Robertson, A.W., Kelly, D. and Ladley, J.J., 2011. Futile selfing in the trees *Fuchsia excorticata* (Onagraceae) and *Sophora microphylla* (Fabaceae): inbreeding depression over 11 years. *International Journal of Plant Sciences*, 172: 191-198.
- Rock, J. F. 1913. The indigenous trees of the Hawaiian Islands. Reprinted in 1974 by Pacific Tropical Botanical Garden, Lawa‘i, Kaua‘i, Hawai‘i, and Charles F. Tuttle, Rutland, Vermont.
- Rojas-Sandoval, J. and Meléndez-Ackerman, E. 2009. Pollination biology of *Harrisia portoricensis* (Cactaceae), an endangered Caribbean species. *American journal of botany*, 96: 2270-2278.
- Sakai, A. K., Wagner, W. L. and Mehrhoff, L. A. 2002. Patterns of Endangerment in the Hawaiian Flora. *Systematic Biology*, 51: 276–302.
- Skaug, H., Fournier, D., Nielsen, A., Magnusson, A., and Bolker, B. 2013. Generalized Linear Mixed Models using AD Model Builder, R package version 0.7.5. Web. https://r-forge.r-project.org/scm/viewvc.php/*checkout*/pkg/inst/doc/glmmADMB.pdf?root=glmmadmb Retrieved 11 May, 2018.
- St. John, H. 1956. The relationship between the species of *Erythrina* (Leguminosae) native to Hawaii and Tahiti: I *Pacific Plant Studies* 13. *Webbia*, 11: 293-299.
- Staples, G. and Herbst, D.R. 2005. A tropical garden flora. Bishop Museum Press.
- Sylva, M. 2014. Effects of fountain grass and ungulate fencing on the health and natural regeneration of wiliwili trees. University of Hawaii at Manoa, Web. <http://hdl.handle.net/10125/33938>. Retrieved 11, March 2017.

- Trauernicht, C., Pickett, E., Giardina, C.P., Litton, C.M., Cordell, S., Beavers, A. 2015. The contemporary scale and context of wildfire in Hawai'i. *Pacific Science*. 69: 427–444.
- Traveset, A. and Richardson, D.M. 2006. Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology and Evolution*, 21: 208–216.
- Venables, W. N. and Ripley, B. D. 2002. *Modern Applied Statistics with S*. Fourth Edition. Springer, New York. ISBN 0-387-95457-0.
- Vicens, N. and Bosch, J. 2000. Weather-dependent pollinator activity in an apple orchard, with special reference to *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae and Apidae). *Environmental Entomology*, 29: 413-420.
- Wagner, W.L., Herbst, D.R. and Sohmer, S.H. 1999. *Manual of the Flowering Plants of Hawai'i*, Vols. 1 and 2 (Rev. ed.). University of Hawai'i and Bishop Museum Press.
- Ziegler, A.C. 2002. *Hawaiian natural history, ecology, and evolution*. University of Hawaii Press.